IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ATTACH TO #4

In re the application of: Gary S. Gray, Jerry Carson, Kashi Javaherian, Paul D. Rennert and Sandra Silver

Group Art Unit: 1642

Examiner: Helms, L.

Serial No.: 09/227,595 Continuation of

Filed: ~~January 8, 1999~~ December 20, 2001

For: CTLA4-Cγ4 Fusion Proteins (as amended)

Attorney Docket No.: RPN-001

Assistant Commissioner for Patents
Washington, D.C. 20231

Certificate ~~of First Class Mailing~~ (37 CFR ~~1.8(a)~~) under 1.10

I hereby certify that this correspondence is being deposited with the United States Postal Service as ~~first class mail~~ "Express Mail to Addressee" in an envelope addressed to: ~~Assistant~~ Commissioner for Patents, Box Patent Application, Washington, D.C. 20231 on the date set forth below.

December 20, 2001
Date of Signature and of Mail Deposit

By: Larry Taylor
~~Megan E. Williams~~ Larry Taylor
~~Registration No. 43,270~~
~~Attorney for Applicants~~

Mailing Label No. EL 833159141US

DECLARATION UNDER 37 C.F.R. 1.131 BY

GARY S. GRAY, JERRY CARSON, KASHI JAVAHERIAN, PAUL D. RENNERT

AND SANDRA SILVER

Sir:

We, Gary S. Gray, Jerry Carson, Kashi Javaherian, Paul D. Rennert And Sandra Silver declare:

1. We are the inventors of the subject matter described and claimed in the above-referenced patent application.

2. Prior to July 11, 1994, the invention described and claimed in the above-referenced application was completed in this country, as evidenced by the following:

A. Prior to July 11, 1994, we were in possession of a cloned CTLA4Ig molecule which was producing large amounts of active protein in CHO DG44 cells.

Page A-1 of Appendix A describes amino acid modifications to the Ig portion of the CTLA4Ig molecule. Deletion of the CH_2 domain from γ1 and mutations to amino acids 235 and 237 in γ4 are described.

Page A-2 of Appendix A describes amplification of the mutated γ4 Hinge-CH_2-CH_3 region and the cloning of the mutated γ4 into pNRDSH/hCTLA4 to replace the existing γ1 Hinge-CH_2-CH_3.

Page A-3 of Appendix A describes a second method which involves the use of nested PCR to generate a mutated γ1 (having an L to A substitution at amino acid 234, an L to E substitution at amino acid 235, and a G to A change at amino acid 237) from hCTLA4Ig and the cloning of the mutated γ1 back into hCTLA4 pNRDSH.

Pages A-5 and A-6 show primers for use in making mutated forms of CTLA4Ig.

The notebook pages submitted as pages A-1 through A-7 of Appendix A demonstrate that we completed the claimed invention in this country prior to July 11, 1994.

B. In addition, as shown on page A-8 of Appendix A, prior to July 11, 1994, we demonstrated that mutated forms of CTLA4Ig effectively competed with biotinylated CTLA4-Ig for binding to B7.
In the experiment presented at the bottom of the page B7Ig was put onto plates. Biotinylated CTLA4Ig was added to the plates in a fixed amount. Test forms of CTLA4Ig were added in serial dilutions to test for their ability to compete for binding to B7 on the plate. In this type of assay as the amount of an effective competitor is increased, the amount of biotinylated CTLA4Ig that binds decreases. This leads to a decrease in the optical density readout. Unlabelled CTLA4Ig (left row) was used as a positive control.
The data show that samples #2, #3, and #4 (in rows 2, 4, and 5, respectively) effectively competed with unmodified CTLA4Ig for binding to B7. As indicated in the table in the middle of the page, sample 2 was oncoCTLA4-mγ4; sample 3 was IgLCTLA4-γ1; and sample 4 was IgLCTLA4-γ1.

PTO/SB/96 (4-04)

These data demonstrate that the modified CTLA4Ig molecules were still able to bind to B7.

Each of the dates deleted from pages A-1 through A-8 of Appendix A are prior to July 11, 1994.

We have been warned that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 or the United States Code, and that such willful and false statements may jeopardize the validity of the subject application or any patent resulting therefrom, and declare that all statements made of our own knowledge are true and that all statements made on information and belief are believed to be true.

Date: Oct. 4, 2001 Signed: Gary S. Gray

Gary S. Gray

Date: ________ Signed: ________

Jerry Carson

Date: ________ Signed: ________

Kashi Javaherian

Date: ________ Signed: ________

Paul D. Rennert

Date: ________ Signed: ________

Sandra Silver

10022075.122001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: Gary S. Gray et al. Serial No.: 08/595,590 Filed: February 2, 1996 For: CTLA4-Immunoglobulin Fusion Proteins Having Modified Effector Functions and Uses Therefor Attorney Docket No.: RPI-007	Group Art Unit: 1806 Examiner: Eyler, Y.

Assistant Commissioner for Patents
Washington, D.C. 20231

Certificate of First Class Mailing (37 CFR 1.8(a))

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231 on the date set forth below.

2-20-98
Date of Signature and of Mail Deposit

By: Giulio DeConti
Giulio A. DeConti, Jr.
Reg. No. 31,503

ASSOCIATE POWER OF ATTORNEY

Sir:

The undersigned attorney has the power of attorney in the subject application. He hereby grants an associate power to:

Megan E. Williams, Ph.D.
Registration No. P43,270
Lahive & Cockfield, LLP
28 State Street
Boston, MA 02109

FEB 25 1998

Please continue to forward all written and telephonic communications to Amy E. Mandragouras at the address and telephone number listed below.

Respectfully submitted,

Giulio A. DeConti, Jr.
Registration No. 31,503
Attorney for Applicants

LAHIVE & COCKFIELD, LLP
28 State Street
Boston, MA 02109
Tel. (617) 742-4214

Dated: February 20, 1998

40025.4200

human - CD4 : Ig

STATUS : This clone, which is currently producing large amounts of active protein in CHODG44, is being methotrexate amplified.

Recently focus has shifted to the Ig tail portion of this clone. Both Fc receptor and complement activation activities are determined by sequence in CH_2 domain.

REFS:

Canfield + Morrison, 1991 J Exp Med (173) 4..
Jung et al, 1991 J Immunol. (147)
Tao et al, 1991 J Exp Med (173) 102..
Duncan + Winter, 1988 Nature (332)

It would be useful to design a construct in which these Ig activities were eliminated.

This work will be exactly analogous to what was done last year for the antibody engineering project, when I deleted the CH_2 domain from γ1 and mutated residues 235 and 237 in γ4

See p 115, Book 1139 and //

See p 52, Book 1177 and //

Read and understood by me   Date

[signature]

T0022T.52020DT

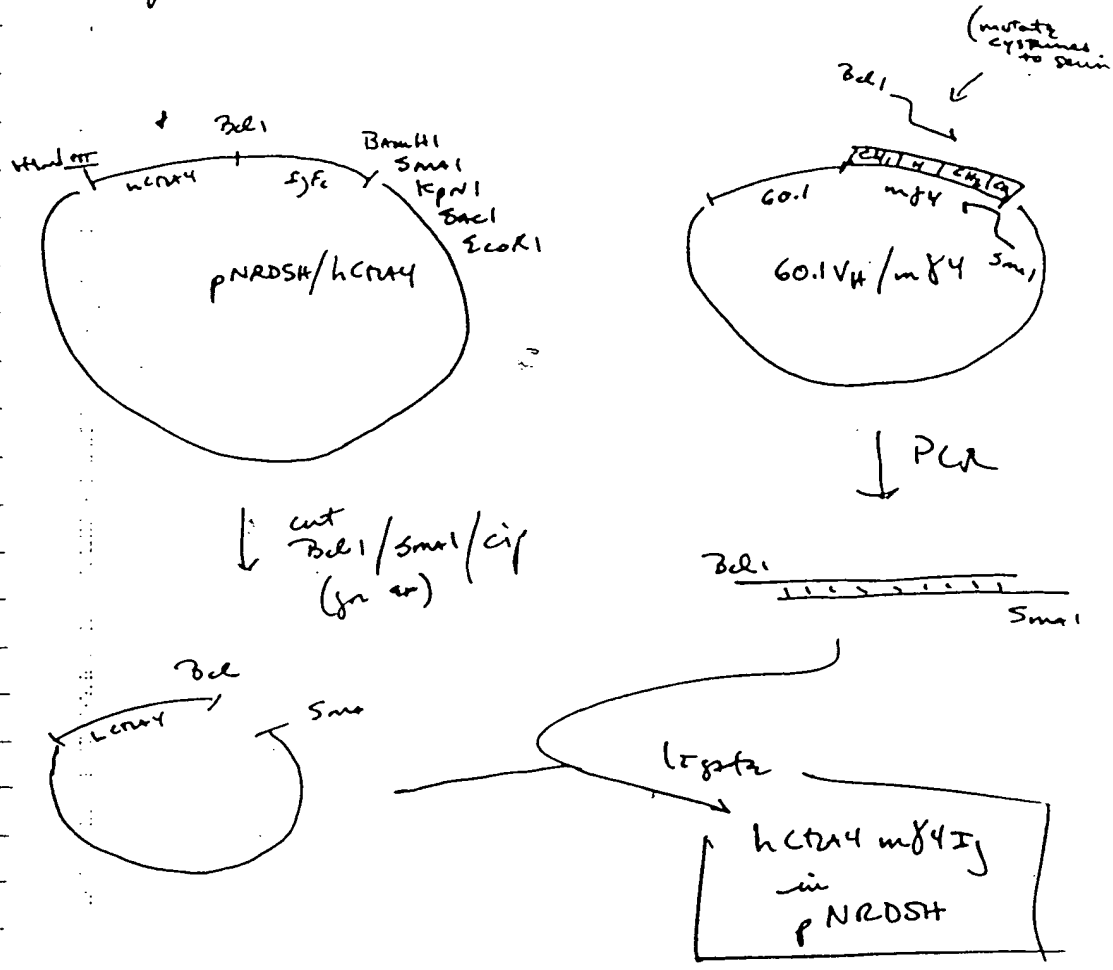
2 STRATEGIES will BE USED:

hCγ4, mutagenesis of IgFc

possible strategies:

① PCR out the mutated γ4 H-CH_2-CH_3 region from 60.1 V_H and clone into pNRDSH/hCγ4 in place of the existing γ_1 H-CH_2-CH_3

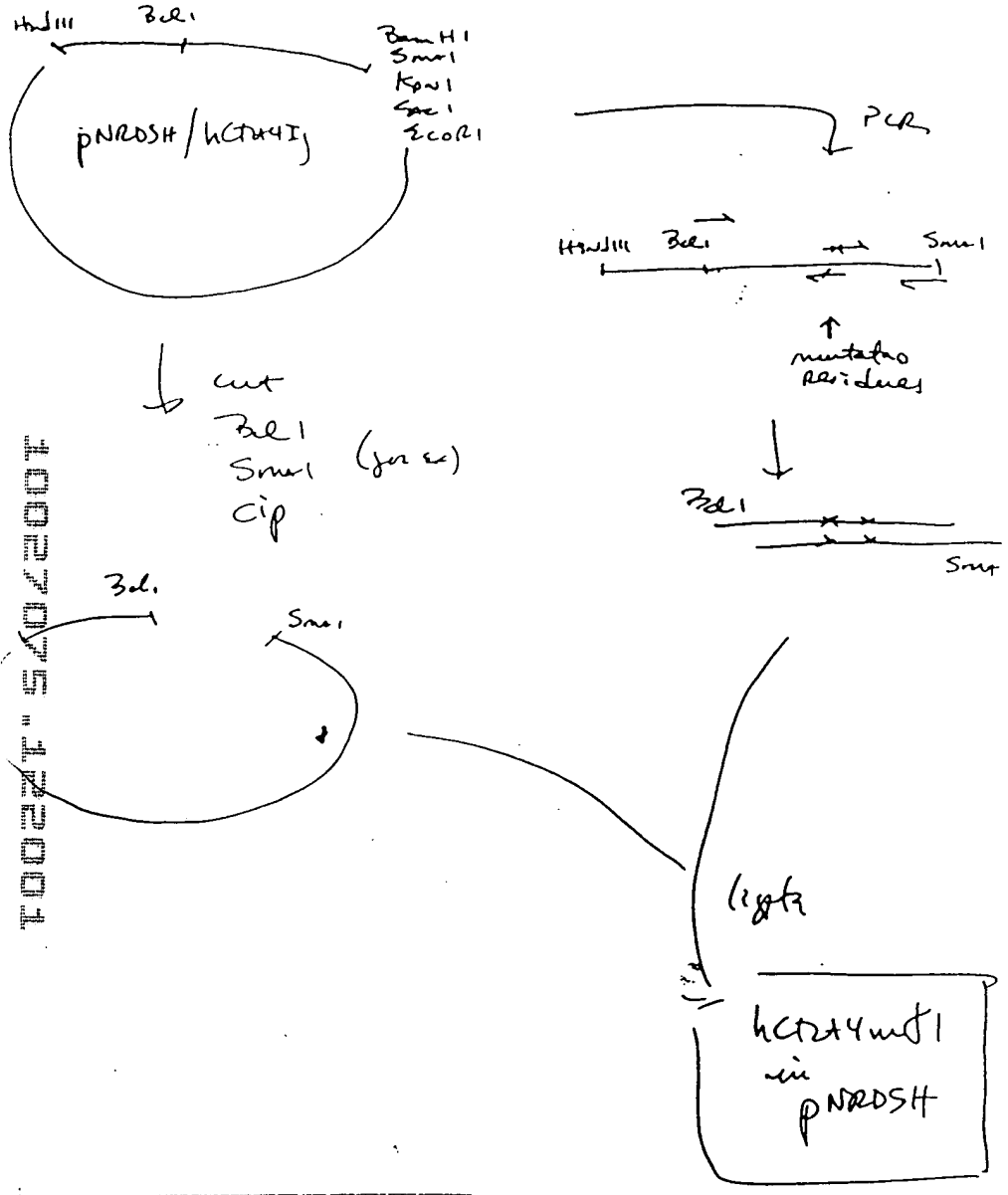
(Note that γ4 also lacks any ability to activate complement - S. Silver)



Read and understood by me

Date

use NESTED PCR to gener - a mutated 81 from hCTLA4Ij . Clone the mt81 back into hCTLA4 ___M = pNRDSH :



For this clone mutate residues as follows:

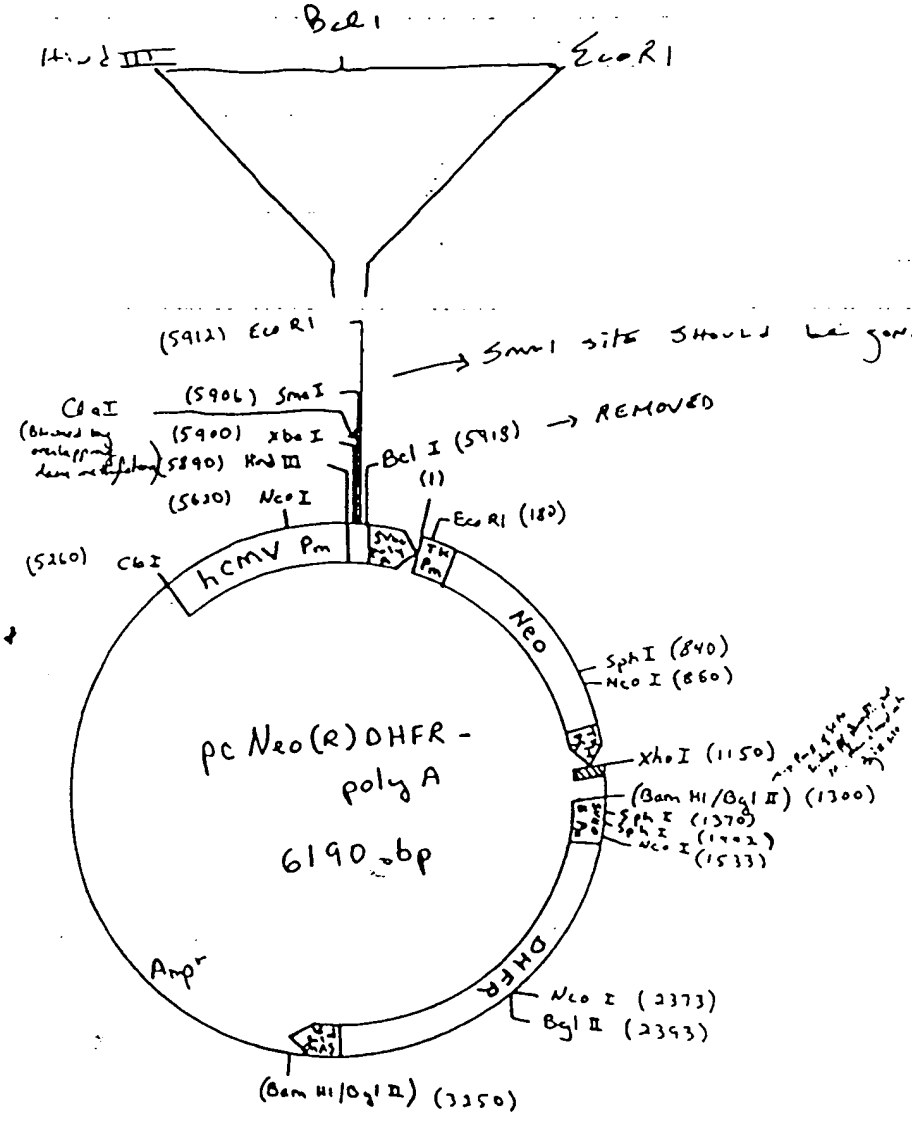
234	L	→ A
235	L	→ E
236	G	
237	G	→ A

Read and understood by me

Date

A-4

Vector:



preproinsulin poly A

Enzymes that DO NOT CUT

EcoRV 1227 p3
SpeI 1227 p3
KpnI (1-9-92)

5

Read and understood by me

Date

4025795-4025804

primers for [redacted] γFc mutagenesis

A-5

for γ4:

5' primer — use G. Gray's original idea to knock out the cysteines in the hinge (γ4 has two)

P D (Q) E S K Y
BCL1
5' GAG CAT TTT CCT GAT CAG GAG TCC AAA TAT

G P P S P S S P
GGT CCC CCA TCC CCA TCA TCC CCA

sequence → (G) (K) (P) (T)
GGT AAG CCA ACCC

DOUBLE CHECK THAT pNRDSH LACKS THIS restriction site

1st use this ↓

3' primer

if NEEDED still have these → →

5' GCAGAG GAATTC GAGCTC GGTACCC GGGGATCC

lock R1 SacI KpnI XmaI BamHI

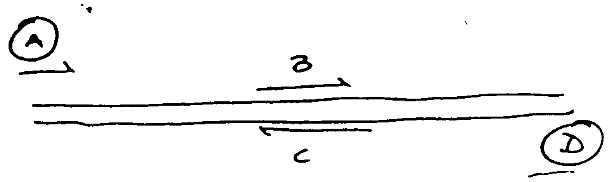
γ4
me CCAGTGTGGGGACAGTGGGACCCGCTCTGCCTCCC 3'

Read and understood by me Date

10022415702 2001

A-6

Fragment



5' primer ✓

(A): use Gary Gray's original γ1 primer:

PRIMER

P BclI Q / E P K S S D K T

5' GAG CAT TTT CCT GAT CAG GAG CCC AAA TCT TCT GAC AAA ACT

H T S P P S P G K

CAC ACA TCT CCA CCG TCT CCA GGT AAA C — IgFc —

—— * —— BamHI - SmaI - KpnI - SacI - EcoRI - ClaI - EcoRV - BglII —

— T7 promoter

3' primer (D):

XmaI / BamHI SmaI KpnI SacI EcoRI

pSP72 MCS: 5' GGATCCCC GGGTACC GAGCTC GAATTC 3'

3' CCTAGGGG CCCATGG CTCGAG CTTAAG 5'

PRIMER:

5' GCAGAGGAATTCGAGCTCGGGTACCCGGGGATCC

lock

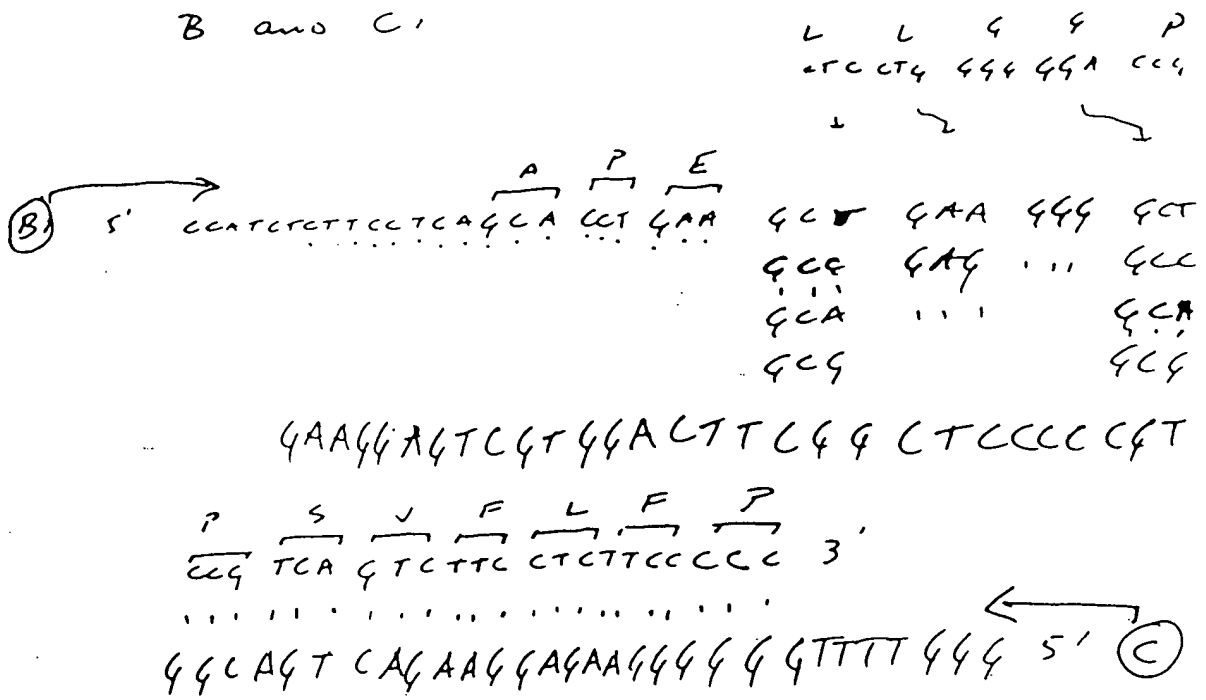
Read and understood by me

Date

100224.5702004

1111 A-7

B and C'



Oligonucleotide Requests

DNA SYNTHESIS REQUEST FORM

REQUESTED BY Paul Bennett

PROJECT CHARGED BJ 1 YCT

DATE REQUESTED .

DATE REQUIRED (NO ASAP)

SEQUENCE NAME Mugamma 4 - 5'

LENGTH 67

SEQUENCE:

pdT

5' GAGCATTTTCCTGATCAGGA
GTCCAAATATGGTCCCCCATT
CCCCATCATCCCCAGGTAAG
CCAAACCC 3'

Read and understood by me

Date

Nicholas Marks

Transient Expression of IgL hCTLA4(3) Ig / EE12 A-8

→ 38

293 culture supernatant tested again a IgG1, IgG4 ELISA using higher dilution.

Results:

DATE:

293 TRANSIENTS

SAMPLE IDENTIFICATION		ug/mL IgG 1	ug/10^6/72hrs IgG 4	Dilutions 1:10 → 1:2
ILL CTLA4(+2)-γ1	1	2.12	1.77	↓
uncoCTLA4-mγ4	2	14.88	3.23	
IgLCTLA4(-2)-γ1	3	34.26	33.65	
IgL CTLA4(3)-γ1	4	33.9	35.54	↓

(+) Controls 2.25 ug/ml 156 ng/ml

expected: 2 ug/ml 125 ng/ml

These samples were tested for their ability to compete for B7 binding. Assay run by Nancy Green.

	Unlabeled CTLA4Ig 1	JC samples #1 2	#2 3	#3 4	#4 5	WT A29 6/16 6	7/13 7	WT A29L 8/13 8	Avg A104 9	Avg A104 10	11	12
70 ug/ml A	0.119	0.404	0.078	0.066	0.063	0.134	0.128	0.150	0.253	0.458		
25 B	0.170	0.412	0.083	0.083	0.075	0.128	0.147	0.182	0.291	0.343		
12.5 C	0.209	0.349	0.096	0.090	0.076	0.153	0.192	0.173	0.323	0.318		
31.25 D	0.287	0.412	0.138	0.104	0.096	0.199	0.237	0.233	0.448	0.398		
5.6 E	0.342	0.450	0.157	0.144	0.122	0.260	0.288	0.282	0.445	0.381		
7.8 F	0.390	0.454	0.268	0.158	0.149	0.285	0.368	0.349	0.549	0.415		
3.9 G	0.384	0.504	0.279	0.198	0.183	0.369	0.462	0.425	0.392	0.408		
0 H	0.425	0.649	0.407	0.462	0.524	0.597	0.496	0.489	0.523	0.424		

(max signal – no inhibitor)

As before the IgLCTLA4 is not functional. The two clones of IgLhCTLA4 do effectively compete w/ CTLA4-Ig – biotinylated.

Plasmids are ready for transfection into stable NSO lines.

→ Samples titrated serially 1:2 – in 50 uL

→ All sample wells contain 50 uL of 70 ng/ml CTLA4Ig biotin

→ 43

Read and understood by me Date

T00227.52042001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: Gary S. Gray, Jerry Carson, Kashi Javaherian, Paul D. Rennert and Sandra Silver	Group Art Unit: 1642
Serial No.: ~~09/227,595~~ Continuation of 09/227,595	Examiner: Helms, L.
Filed: J~~anuary 8, 1999~~ December 20, 2001	
For: CTLA4-Cγ4 Fusion Proteins (as amended)	
Attorney Docket No.: RPN-001	

MEW

Assistant Commissioner for Patents
Washington, D.C. 20231

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December 20, 2001
Date of Signature and of Mail Deposit

By: Larry Taylor
~~Megan E. Williams~~ Larry Taylor
~~Registration No. 43,270~~
Att~~orney for Applic~~ants

Mailing Label No. EL 833315914US

MEW

DECLARATION UNDER 37 C.F.R. 1.131 BY

GARY S. GRAY, JERRY CARSON, KASHI JAVAHERIAN, PAUL D. RENNERT AND SANDRA SILVER

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2. Prior to July 11, 1994, the invention described and claimed in the above-referenced application was completed in this country, as evidenced by the following:

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The notebook pages submitted as pages A-1 through A-7 of Appendix A demonstrate that we completed the claimed invention in this country prior to July 11, 1994.

B. In addition, as shown on page A-8 of Appendix A, prior to July 11, 1994, we demonstrated that mutated forms of CTLA4Ig effectively competed with biotinylated CTLA4-Ig for binding to B7.
In the experiment presented at the bottom of the page B7Ig was put onto plates. Biotinylated CTLA4Ig was added to the plates in a fixed amount. Test forms of CTLA4Ig were added in serial dilutions to test for their ability to compete for binding to B7 on the plate. In this type of assay as the amount of an effective competitor is increased, the amount of biotinylated CTLA4Ig that binds decreases. This leads to a decrease in the optical density readout. Unlabelled CTLA4Ig (left row) was used as a positive control.
The data show that samples #2, #3, and #4 (in rows 2, 4, and 5, respectively) effectively competed with unmodified CTLA4Ig for binding to B7. As indicated in the table in the middle of the page, sample 2 was oncoCTLA4-mγ4; sample 3 was IgLCTLA4-γ1; and sample 4 was IgLCTLA4-γ1.

FEB 25 2004 14:02

These data demonstrate that the modified CTLA4Ig molecules were still able to bind to B7.

Each of the dates deleted from pages A-1 through A-8 of Appendix A are prior to July 11, 1994.

We have been warned that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 or the United States Code, and that such willful and false statements may jeopardize the validity of the subject application or any patent resulting therefrom, and declare that all statements made of our own knowledge are true and that all statements made on information and belief are believed to be true.

Date: _____ Signed: _____

Gary S. Gray

Date: October 4, 2001 Signed: _____

Jerry Carson

Date: _____ Signed: _____

Kashi Javaherian

Date: _____ Signed: _____

Paul D. Rennert

Date: _____ Signed: _____

Sandra Silver

FOIA-5-20-2004

human - CTLA4 : Ig

STATUS: This clone, which is currently producing large amounts of active protein in CHODG44, is being methotrexate amplified.

Recently focus has shifted to the Ig tail portion of this clone. Both Fc receptor and complement activation activities are determined by sequence in CH_2 domain.

REFS: Canfield + Morrison, 1991 J Exp Med (173) 4..
Jung et al, 1991 J Immunol. (147)
Tao et al, 1991 J Exp Med (173) 102
Duncan + Winter, 1988 Nature (332)

It would be useful to design a construct in which these Ig activities were eliminated.

This work will be exactly analogous to what was done last year for the antibody engineering project, where I deleted the CH_2 domain from γ1 and mutated residues 235 and 237 in γ4.

See p 115, Book 1139 and //
See p 52, Book 1177 and //

Read and understood by me

Date

100221.522001

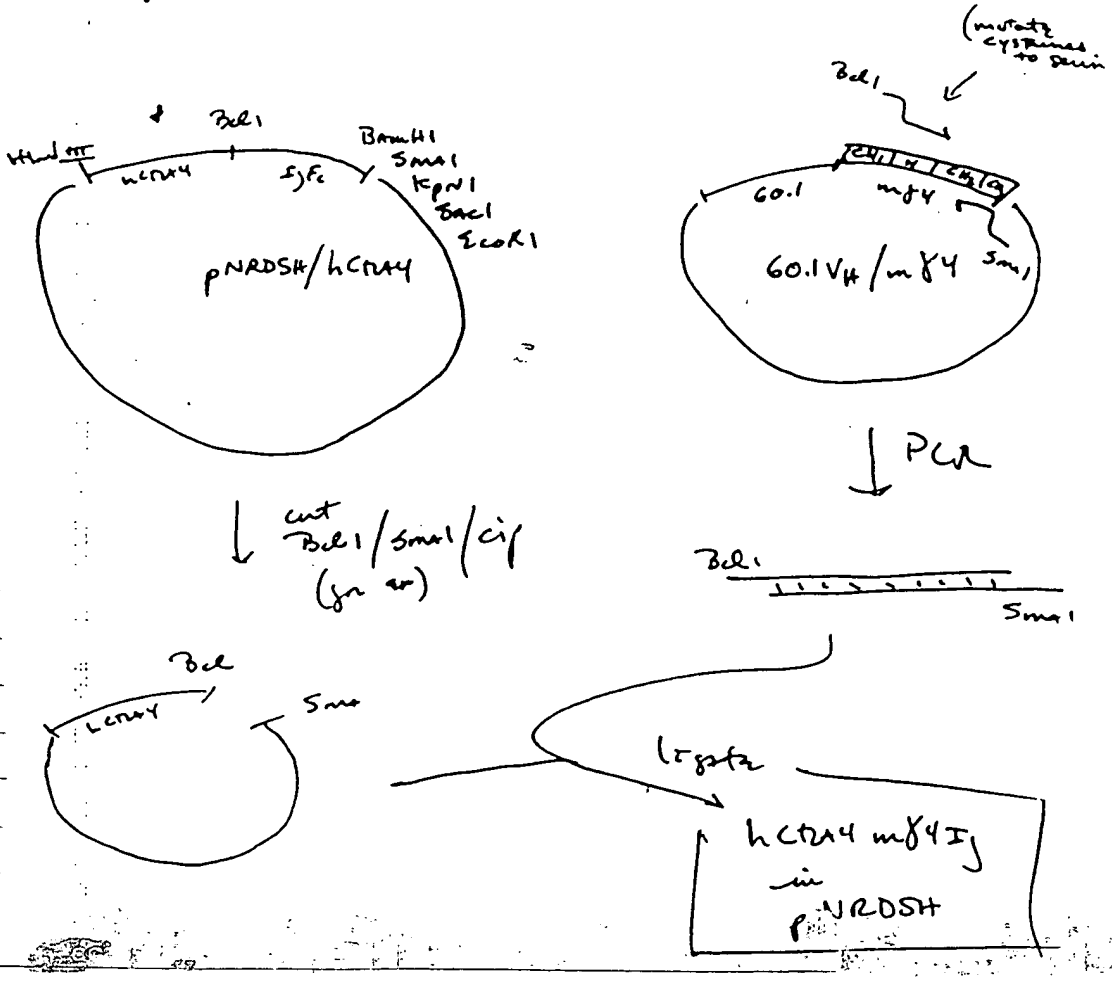
2 STRATEGIES will be USED:

hCγ4, mutagenesis of J_HC

possible strategies:

① PCR out the mutated γ4 $H-CH_2-CH_3$ region from 60.1 V_H and clone into pNRDSH/hCγ4 in place of the existing γ_1 $H-CH_2-CH_3$

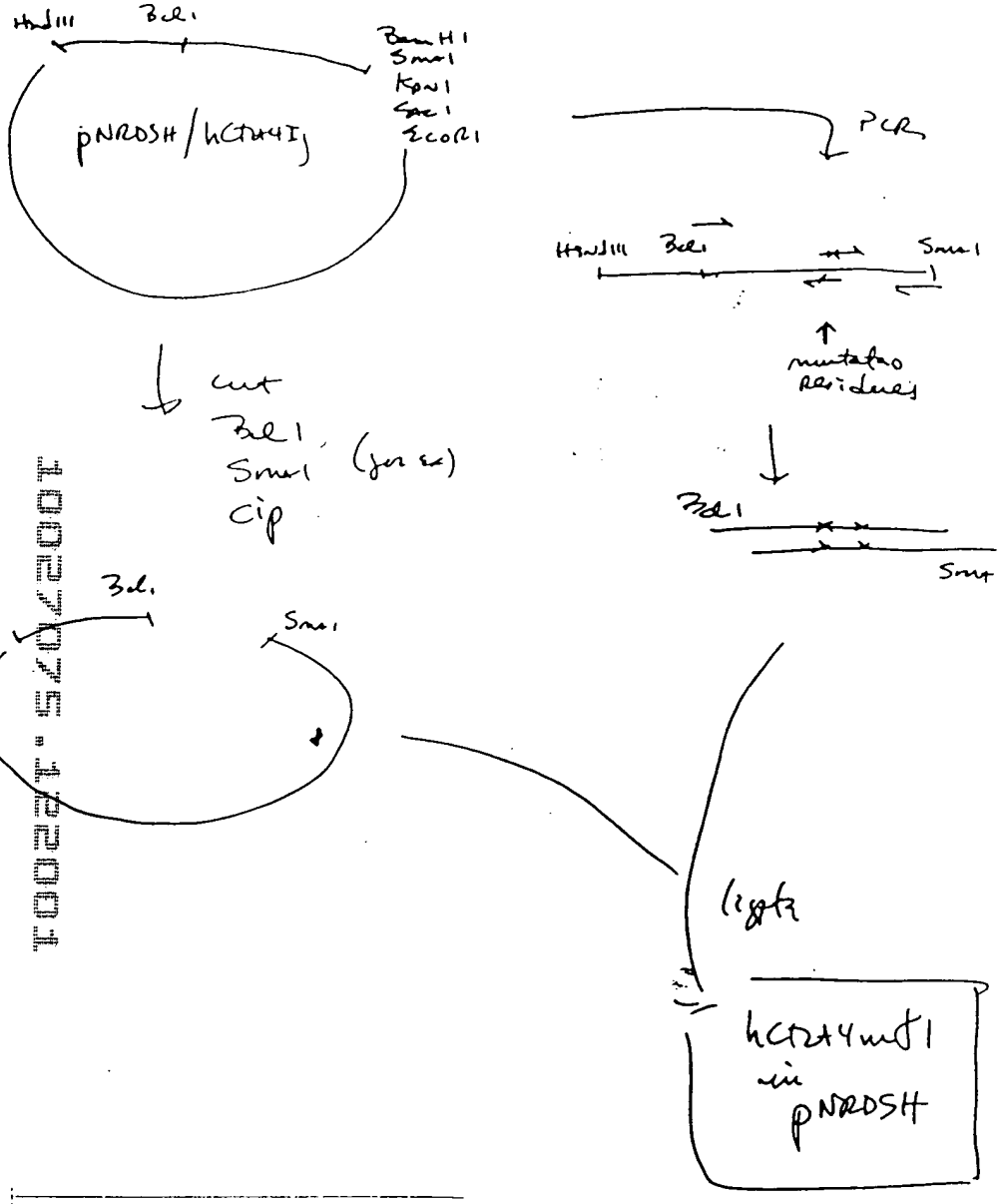
(Note that γ4 also lacks any ability to activate complement - S. Silver)



Read and understood by me

Date

USE NESTED PCR to generate a mutated S1 from [redacted] hCTLA4 Ig. Clone this mS1 back into hCTLA4 [redacted] M pNRDSH:



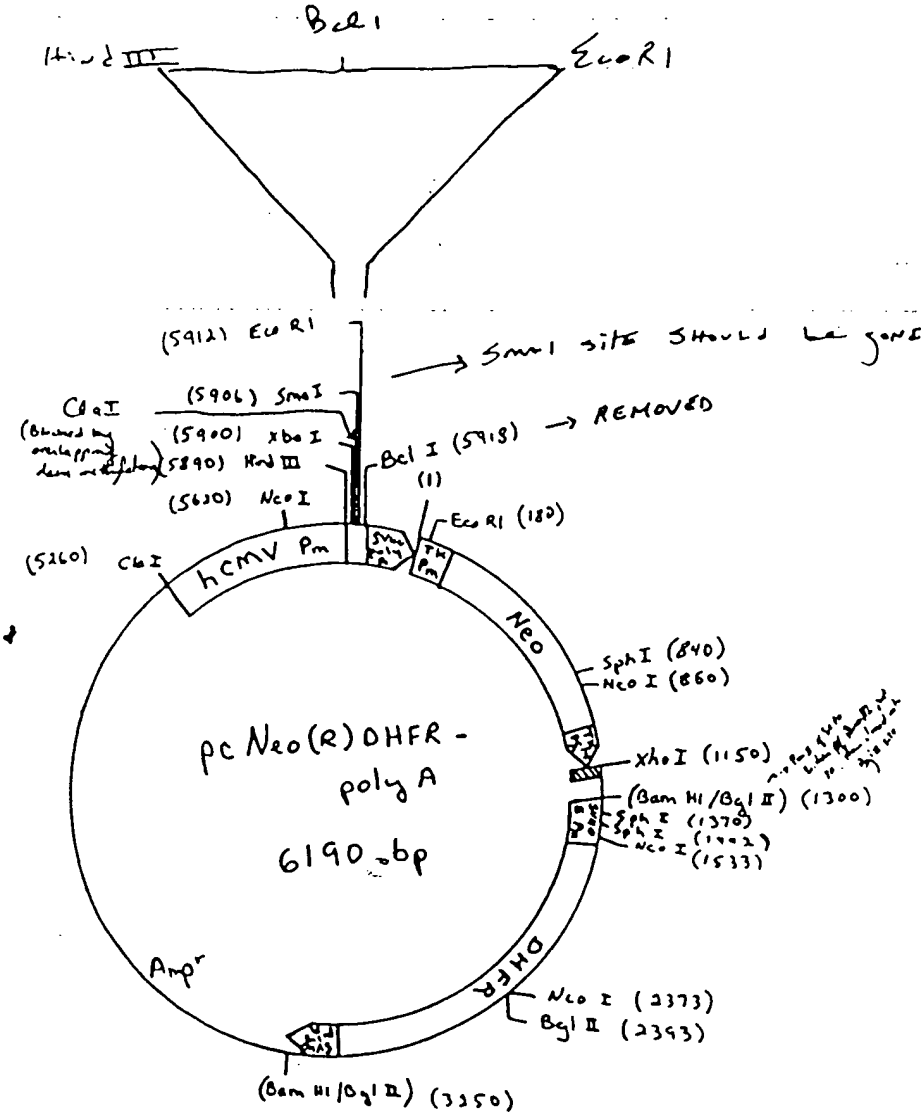
For this clone mutated residues as follows:

234	L	→ A
235	L	→ E
236	G	
237	G	→ A

Read and understood by me

Date

Vector:



Enzymes that DO NOT CUT

EcoRV 1227 p3
SpeI 1227 p3
KpnI (4-9-92)

Read and understood by me

Date

primers for [redacted] Fc mutagenesis

for γ4:

5' primer - use G. Gray's original idea to knock out the cysteines in the hinge (γ4 has two)

P D (Q) E S K Y

BCL 1

5' GAG CAT TTT CCT GAT CAG GAG TCC AAA TAT

G P P S P S S P

GGT CCC CCA TCC CCA TCA TCC CCA

(G) (K) (P) (T)

GGT AAG CCA ACCC

DOUBLE CHECK THAT NRDSH LACKS THIS restriction site

1st use this

3' primer

if needed still have these →

5' GCAGAG GAATTC GAG CTC GGTACCC GGGGATCC

lock R1 Sac1 KpN1 Xma1 BamH1

CCAGTGTGGGG ACAG TGGG A CC CGCTCT GCC TCCC

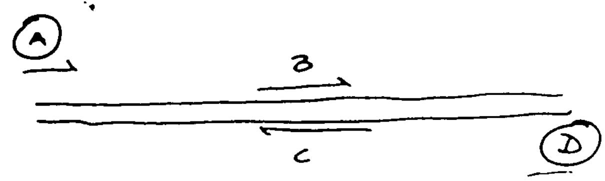
3'

Read and understood by me

Date

[redacted]

A-6



5' primer ✓

(A): use Gary Gray's original γ₁ primer:

PRIMER

5' GAG CAT TTT CCT GAT CAG GAG CCC AAA TCT TCT GAC AAA ACT

CAC ACA TCT CCA CCG TCT CCA GGT AAA C — IgFc —

—— * —— BamHI - SmaI - KpnI - SacI - EcoRI - ClaI - EcoRV - BglII —

— T7 promoter

3' primer (D):

pSP72 MCS:

BamHI / XmaI SmaI / KpnI / SacI / EcoRI

5' GGATCCCC GGG TACC GAG CTC GA ATTC 3'

3' CCTAGGGG CCC ATGG CTC GAG CTTAAG 5'

PRIMER:

5' GCAGAG GAATTCGAG CTC GGTACCCGGGGATCC

lock

Read and understood by me

Date

1111 A-7

B and C:

L L G G P
CTC CTG GGG GGA CCG

(B) 5' CCATCTCTTCCTCAGCA CCT GAA GCT GAA GGG GCT
GCG GAG ... GCC
GCA ... GCA
GCG GCG

GAAGGAGTCGTGGACTTCGG CTCCCC CGT

P S V F L F P
CCG TCA GTC TTC CTC TTC CCC C 3'
... ...
GGCAGT CAG AAG GAG AAGGGGG G GTTTT GGG 5' (C)

Oligonucleotide Requests

DNA SYNTHESIS REQUEST FORM

REQUESTED BY Paul Bunnell

PROJECT CHARGED BJ # 967

DATE REQUESTED

DATE REQUIRED (NO ASAP)

SEQUENCE NAME muGAMMA4 - 5'

LENGTH 67

SEQUENCE:

pdf

5' GAGCATTTTTCCTGATCAGGA
GTCCAAAATATGGTCCCCATT
CCCCATCATCCCCAGGGTAAG
CCAACCC 3'

Read and understood by me

Date

A-8

Transient Expression of IgLhCTLA4 3 Ig / EE12

→ 3F

293 culture supernatant tested again a IgG1, IgG4 ELISA using higher dilution.

Results:

DATE:

293 Transients

IDENTIFICATION	ug/mL IgG1	IgG4	Dilutions 1:10 → 1:2
ILL CTLA4 (+2) - γ1 1	2.12	1.77	↓
CTLA4 - m γ4 2	14.88	3.23	
IgL CTLA4 (+2) - γ1 3	34.26	33.65	
IgL CTLA4(3) - γ1 4	33.9	35.54	↓

⊕ Controls 2.28 μg/ml 156 ng/ml

expected: 2 μg/ml 125 ng/ml

These samples were tested for their ability to compete for B7 bin. Assay run by Nancy Green.

Optical Density

		unlabelled CTLA4 Ig 1	#1 2	#2 3	#3 4	#4 5	6	7	8	9	10	11	12
70 μg/ml	A	0.119	0.404	0.078	0.066	0.063	0.134	0.128	0.150	0.253	0.458		
25	B	0.170	0.412	0.063	0.083	0.075	0.128	0.147	0.182	0.291	0.343		
12.5	C	0.209	0.349	0.096	0.090	0.076	0.153	0.192	0.173	0.323	0.318		
31.25	D	0.287	0.412	0.138	0.104	0.096	0.199	0.237	0.233	0.448	0.398		
15.6	E	0.342	0.450	0.157	0.144	0.122	0.260	0.288	0.282	0.445	0.381		
7.8	F	0.390	0.454	0.268	0.158	0.149	0.285	0.368	0.349	0.549	0.415		
3.9	G	0.384	0.504	0.279	0.198	0.183	0.369	0.462	0.425	0.392	0.408		
0	H	0.425	0.649	0.407	0.462	0.524	0.507	0.496	0.489	0.523	0.424		

As before the IgLhCTLA4 is not functional. The two clones of IgLhCTLA4 do effectively compete w/ CTLA4-Ig-biotinylated.

Plasmids are ready for transfection into stable NS0 lines.

→ Samples titered serially 1:2 - in 50 μl vol

→ All sample wells contain 50 μl of 70 ng/ml CTLA4 Ig biotin

→ 43

Read and understood by me Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: Gary S. Gray, Jerry Carson, Kashi Javaherian, Paul D. Rennert and Sandra Silver Continuation of NEW Serial No.: 09/227,595 Filed: ~~January 8, 1999~~ December 20, 2001 For: CTLA4-Cγ4 Fusion Proteins (as amended) Attorney Docket No.: RPN-001	Group Art Unit: 1642 Examiner: Helms, L.

Assistant Commissioner for Patents
Washington, D.C. 20231

NEW Certificate ~~of First Class Mailing~~ (37 CFR ~~1.8(a)~~ Under 1.10)

I hereby certify that this correspondence is being deposited with the United States Postal Service as ~~first class mail~~ "Express Mail" to Addressee in an envelope addressed to: ~~Assistant~~ Commissioner for Patents, Box Patent Application, Washington, D.C. 20231 on the date set forth below.

December 20, 2001
Date of Signature and of Mail Deposit

By: Larry Taylor
~~Megan E. Williams~~
~~Registration No. 43,270~~
~~Attorney for Applicants~~

Mailing Label No. EL833139141US

NEW

DECLARATION UNDER 37 C.F.R. 1.131 BY

GARY S. GRAY, JERRY CARSON, KASHI JAVAHERIAN, PAUL D. RENNERT AND SANDRA SILVER

Sir:

We, Gary S. Gray, Jerry Carson, Kashi Javaherian, Paul D. Rennert And Sandra Silver declare:

1. We are the inventors of the subject matter described and claimed in the above-referenced patent application.

2. Prior to July 11, 1994, the invention described and claimed in the above-referenced application was completed in this country, as evidenced by the following:

A. Prior to July 11, 1994, we were in possession of a cloned CTLA4Ig molecule which was producing large amounts of active protein in CHO DG44 cells.

Page A-1 of Appendix A describes amino acid modifications to the Ig portion of the CTLA4Ig molecule. Deletion of the CH_2 domain from γ1 and mutations to amino acids 235 and 237 in γ4 are described.

Page A-2 of Appendix A describes amplification of the mutated γ4 Hinge-CH_2-CH_3 region and the cloning of the mutated γ4 into pNRDSH/hCTLA4 to replace the existing γ1 Hinge-CH_2-CH_3.

Page A-3 of Appendix A describes a second method which involves the use of nested PCR to generate a mutated γ1 (having an L to A substitution at amino acid 234, an L to E substitution at amino acid 235, and a G to A change at amino acid 237) from hCTLA4Ig and the cloning of the mutated γ1 back into hCTLA4 pNRDSH.

Pages A-5 and A-6 show primers for use in making mutated forms of CTLA4Ig.

The notebook pages submitted as pages A-1 through A-7 of Appendix A demonstrate that we completed the claimed invention in this country prior to July 11, 1994.

B. In addition, as shown on page A-8 of Appendix A, prior to July 11, 1994, we demonstrated that mutated forms of CTLA4Ig effectively competed with biotinylated CTLA4-Ig for binding to B7.
In the experiment presented at the bottom of the page B7Ig was put onto plates. Biotinylated CTLA4Ig was added to the plates in a fixed amount. Test forms of CTLA4Ig were added in serial dilutions to test for their ability to compete for binding to B7 on the plate. In this type of assay as the amount of an effective competitor is increased, the amount of biotinylated CTLA4Ig that binds decreases. This leads to a decrease in the optical density readout. Unlabelled CTLA4Ig (left row) was used as a positive control.
The data show that samples #2, #3, and #4 (in rows 2, 4, and 5, respectively) effectively competed with unmodified CTLA4Ig for binding to B7. As indicated in the table in the middle of the page, sample 2 was oncoCTLA4-mγ4; sample 3 was IgLCTLA4-γ1; and sample 4 was IgLCTLA4-γ1.

These data demonstrate that the modified CTLA4Ig molecules were still able to bind to B7.

Each of the dates deleted from pages A-1 through A-8 of Appendix A are prior to July 11, 1994.

We have been warned that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 or the United States Code, and that such willful and false statements may jeopardize the validity of the subject application or any patent resulting therefrom, and declare that all statements made of our own knowledge are true and that all statements made on information and belief are believed to be true.

Date: ____________ Signed: ____________

Gary S. Gray

Date: ____________ Signed: ____________

Jerry Carson

Date: 10-3-01 Signed: Kashi Javaherian

Kashi Javaherian

Date: ____________ Signed: ____________

Paul D. Rennert

Date: ____________ Signed: ____________

Sandra Silver

10022705.122001

HUMAN - CTLA4 - Ig

STATUS: This clone, which is currently producing large amounts of protein in CHODG44, is being methotrexate amplified.

Recently focus has shifted to the Ig tail portion of this clone. Both Fc receptor and complement activation activities are determined by sequence in CH_2 domain.

REFS: Canfield + Morrison, 1991 J Exp Med (173) 1483
Jung et al, 1991 J Immunol. (147)
Tao et al, 1991 J Exp Med (173) 1025
Duncan + Winter, 1988 Nature (332)

It would be useful to design a construct in which these Ig activities were eliminated.

This work will be exactly analogous to what was done last year for the antibody engineering project, where I deleted the CH_2 domain from γ_1 and mutated Residues 235 and 237 in γ_4

See p 115, Book 1139 and //

See p 52, Book 1177 and //

Read and understood by me

Date

1002705-1002804

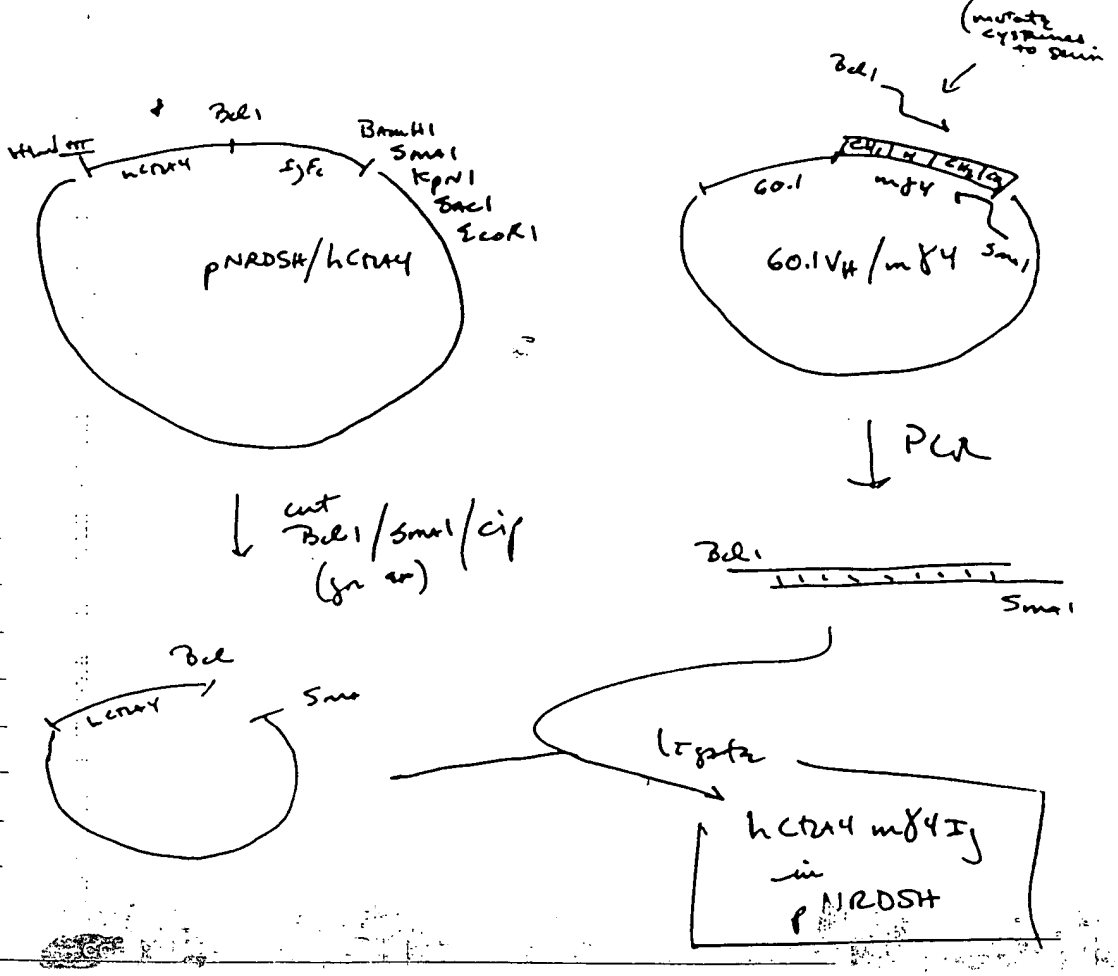
2 STRATEGIES will be USED:

hCγ4, mutagenesis of IgFc

possible strategies:

① PCR out the mutated γ4 H-CH_2-CH_3 region from 60.1 V_H and clone into pNRDSH/hCγ4 in place of the existing γ_1 H-CH_2-CH_3

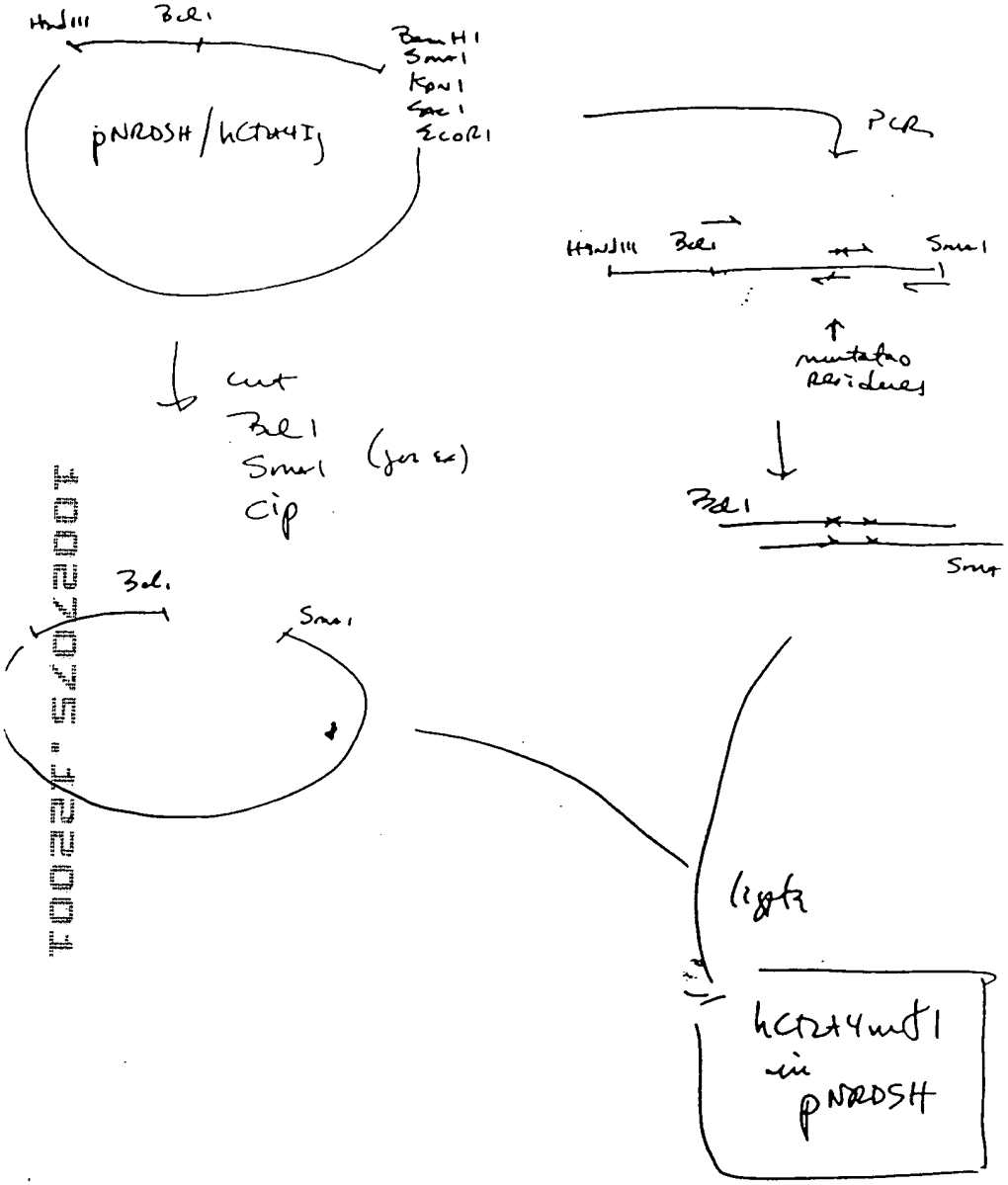
(Note that γ4 also lacks any ability to activate complement - S. Silver)



Read and understood by me

Date

USE NESTED PCR to generate a mutated 81 from hCTLA4Ij. Clone the mt81 back into hCTLA4 M pNRDSH:



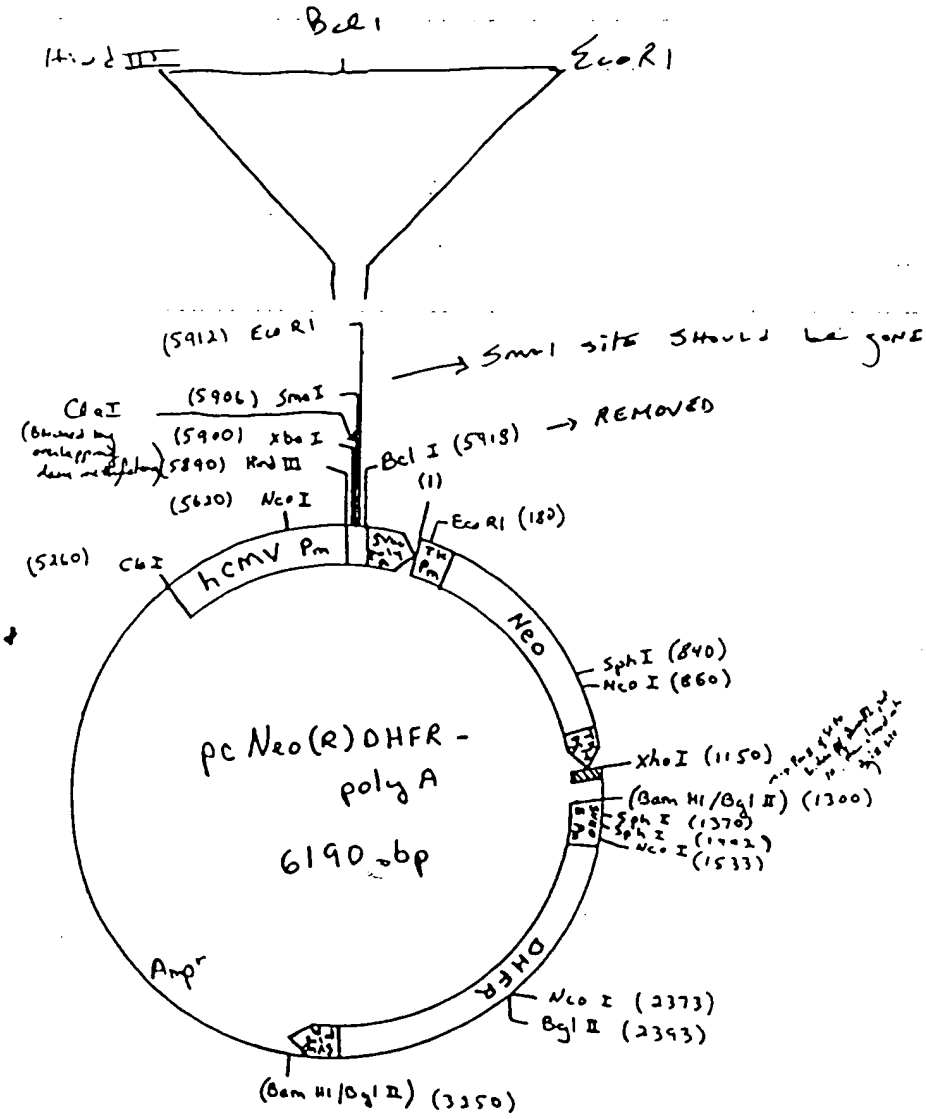
For this clone mutated residues as follows:

234	L	→ A
235	L	→ E
236	G	
237	G	→ A

Read and understood by me

Date

Vector:



preproinsulin poly A

Enzymes that DO NOT CUT

EcoRV 1227 p3
SpeI 1227 p3
KpnI (4-9-92)

5

Read and understood by me

Date

T00224 5/20/2004

primers for γ-Fc mutagenesis

for γ4:

5' primer - use G. Gray's original idea to knock out the cysteines in the hinge (γ4 has two)

P D (Q) E S K Y

BCL 1

5' GAG CAT TTT CCT GAT CAG GAG TCC AAA TAT

G P P S P S S P

GGT CCC CCA TCC CCA TCA TCC CCA

primer → (G) (K) (P) (T)

γ4 GGT AAG CCA ACCC

DOUBLE CHECK THAT pNRDSH LACKS THIS restriction site

3' primer

1st use this

if necessary still have these →

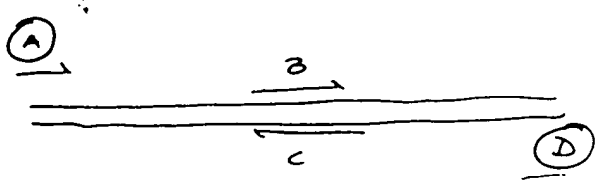
5' GCAGAG GAATTC GAG CTC GGTACCC GGGGATCC

lock R1 SacI KpN1 XmaI BamHI

γ4 mc CCAGTGTGGGG ACAG TGGG A CC CGCTCT GCC TCCC 3'

Read and understood by me Date

Brad R Cass



5' primer ✓

(A): use Gary Gray's original 5' primer:

PRIMER

5' GAG CAT TTT CCT GAT CAG GAG CCC AAA TCT TCT CAC AAA ACT
P Bgl2 Q E Q K S S D K T
CAC ACA TCT CCA CCG TCT CCA GGT AAG C — IgFc —
H T S P P S P G K

— * — BamHI-SmaI-KpnI-SacI-EcoRI-ClaI-EcoRS-Bgl2 —

— T7 promoter

3' primer (D): XmaI

BamHI SmaI KpnI SacI EcoRI

pSB72 MCS: 5' GGATCCCC GGGTACC GAG CTC GA ATTC 3'

3' CCTAGGGG CCCATGG CTC GAG CTTAAG 5'

PRIMER:

5' GCAGAG GAATTCGAG CTC GGTACCCGGGGATCC

lock

Read and understood by me

Date

EQ02LT-5/20/2004

1111

A-7

B and C:

L L G G P
ATC CTG GGG GGA CCG

A P E
(B) 5' CCATCTCTTCCTCAGCA CCT GAA GCT GAA GGG GCT
GCG GAG ... GCC
GCA ... GCA
GCG GCG

GAAGGAGTCGTGGACTTCGG CTCCCC CGT

P S V F L F P
CCG TCA GTC TTC CTC TTC CCC C 3'
... ...
GGCAGT CAG AAG GAG AAG GGG G G GTTTT GGG 5' (C)

Oligonucleotide Requests:

DNA SYNTHESIS REQUEST FORM

REQUESTED BY Paul Bunnell

PROJECT CHARGED 87 # 967

DATE REQUESTED .

DATE REQUIRED (NO ASAP)

SEQUENCE NAME muGAMMA4 - 5'

LENGTH 67

SEQUENCE:

Pst I

5' GAGCATTTTCCTGATCAGGA
GTCCAAAATATGGTCCCCATT
CCCCATCATCCCCAGGGTAAG
CCAACCC 3'

Read and understood by me

Date

A-8

Transient Expression of IgL hCTLA4 Ig / EE12

→ 38

293 culture supernatant tested again a IgG1, IgG4 ELISA using higher dilution.

Results:

DATE:

293 Transients

IDENTIFICATION		ug/mL IgG 1	IgG 4	Dilutions 1:10 → 1:2
ILL CTLA4 (+2) - γ1	1	2.12	1.77	↓
vnco CTLA4 - m84	2	14.88	3.23	↓
IgL CTLA4 (-2) - γ1	3	34.26	33.65	↓
IgL CTLA4(3) - γ1	4	33.9	35.54	↓

⊕ Controls — 2.25 μg/ml — 156 ng/ml

expected: — 2 μg/ml — 125 ng/ml

These samples were tested for their ability to compete for B7 binding assay run by Nancy Green.

Optical Density

		1 unlabeled CTLA4 Ig	2 JC samples #1	3 #2	4 #3	5 #4	6 WT A...	7 ...	8 ...	9 ...	10 ...	11	12
70 μg/ml	A	0.119	0.404	0.078	0.066	0.063	0.134	0.128	0.150	0.253	0.458		
25	B	0.170	0.412	0.083	0.083	0.075	0.128	0.147	0.182	0.291	0.343		
12.5	C	0.209	0.349	0.096	0.090	0.076	0.153	0.192	0.173	0.323	0.318		
31.25	D	0.287	0.412	0.138	0.104	0.096	0.199	0.237	0.233	0.448	0.398		
15.6	E	0.342	0.450	0.157	0.144	0.122	0.260	0.288	0.282	0.445	0.381		
7.8	F	0.390	0.454	0.268	0.158	0.149	0.285	0.368	0.349	0.549	0.415		
3.9	G	0.384	0.504	0.279	0.198	0.183	0.369	0.462	0.425	0.392	0.408		
0	H	0.425	0.649	0.407	0.462	0.524	0.597	0.496	0.489	0.523	0.424		

(max signal)

As before the IgL hCTLA4 is not functional. The two clones of IgL hCTLA4 do effectively compete w CTLA4-Ig-biotinylated.

Plasmids are ready for transfection into stable NSO lines.

→ 43

→ Samples titrated serially 1:2 - in 50 μL

→ All sample wells contain 50 of 70 ng/ml CTLA4 Ig biotin

Read and understood by me

Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: Gary S. Gray, Jerry Carson, Kashi Javaherian, Paul D. Rennert and Sandra Silver Continuation of Serial No.: 09/227,595 new Filed: ~~January 8, 1999~~ December 20, 2001 For: CTLA4-Cγ4 Fusion Proteins (as amended) Attorney Docket No.: RPN-001	Group Art Unit: 1642 Examiner: Helms, L.

Assistant Commissioner for Patents
Washington, D.C. 20231

new Under 1.10

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December 20, 2001
Date of Signature and of Mail Deposit

By: Larry Taylor
~~Megan E. Williams~~
~~Registration No. 43,270~~
~~Attorney for Applicants~~ new

Mailing Label No. EL 933315914US

DECLARATION UNDER 37 C.F.R. 1.131 BY GARY S. GRAY, JERRY CARSON, KASHI JAVAHERIAN, PAUL D. RENNERT AND SANDRA SILVER

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10022705.122001

These data demonstrate that the modified CTLA4Ig molecules were still able to bind to B7.

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Date: ____________________ Signed: ________________________

Gary S. Gray

Date: ____________________ Signed: ________________________

Jerry Carson

Date: ____________________ Signed: ________________________

Kashi Javaherian

Date: 3 October 2001 Signed: [signature]

Paul D. Rennert

Date: ____________________ Signed: ________________________

Sandra Silver

10027075.122001

human - CTLA4 : Ig

STATUS: This clone, which is currently producing large amounts of active protein in CHO DG44, is being methotrexate amplified.

Recently focus has shifted to the Ig tail portion of this clone. Both Fc receptor and complement activation activities are determined by sequence in CH2 domain.

REFS:
Canfield + Morrison, 1991 J Exp Med (173) 4
Jung et al, 1991 J Immunol. (147)
Tao et al, 1991 J Exp Med (173) 02
Duncan + Winter, 1988 Nature (332)

It would be useful to design a construct in which these Ig activities were eliminated.

This work will be exactly analogous to what was done last year for the antibody engineering project, where I deleted the CH2 domain from γ1 and mutated residues 235 and 237 in γ4.

See p 115, Book 1139 and //

See p 52, Book 1177 and //

Read and understood by me

Date

100227075.122001

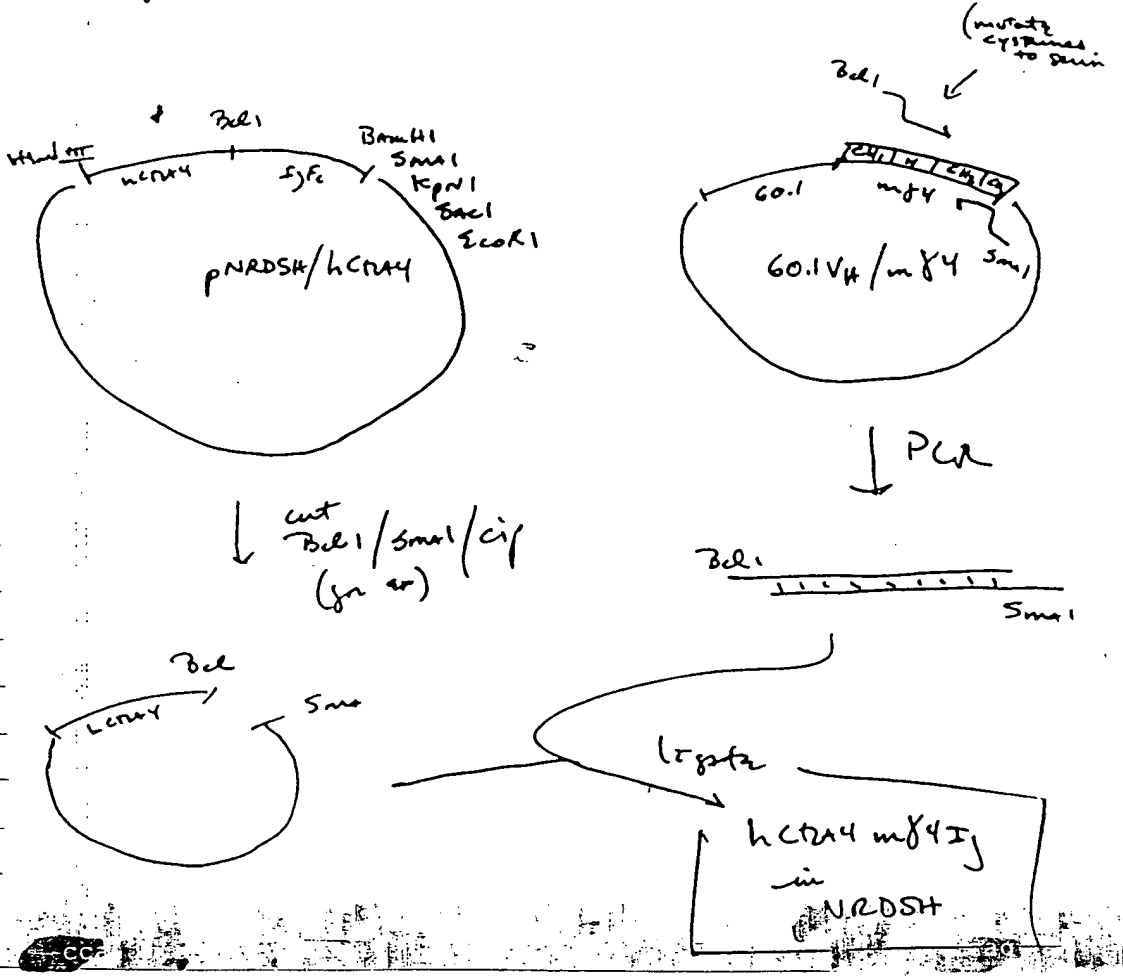
2 STRATEGIES will be USED:

hCγ4, mutagenesis of I_gFc

possible strategies:

① PCR out the mutated γ4 $H-CH_2-CH_3$ region from 60.1 V_H and clone into pNRDSH/hCγ4 in place of the EXISTING γ_1 $H-CH_2-CH_3$

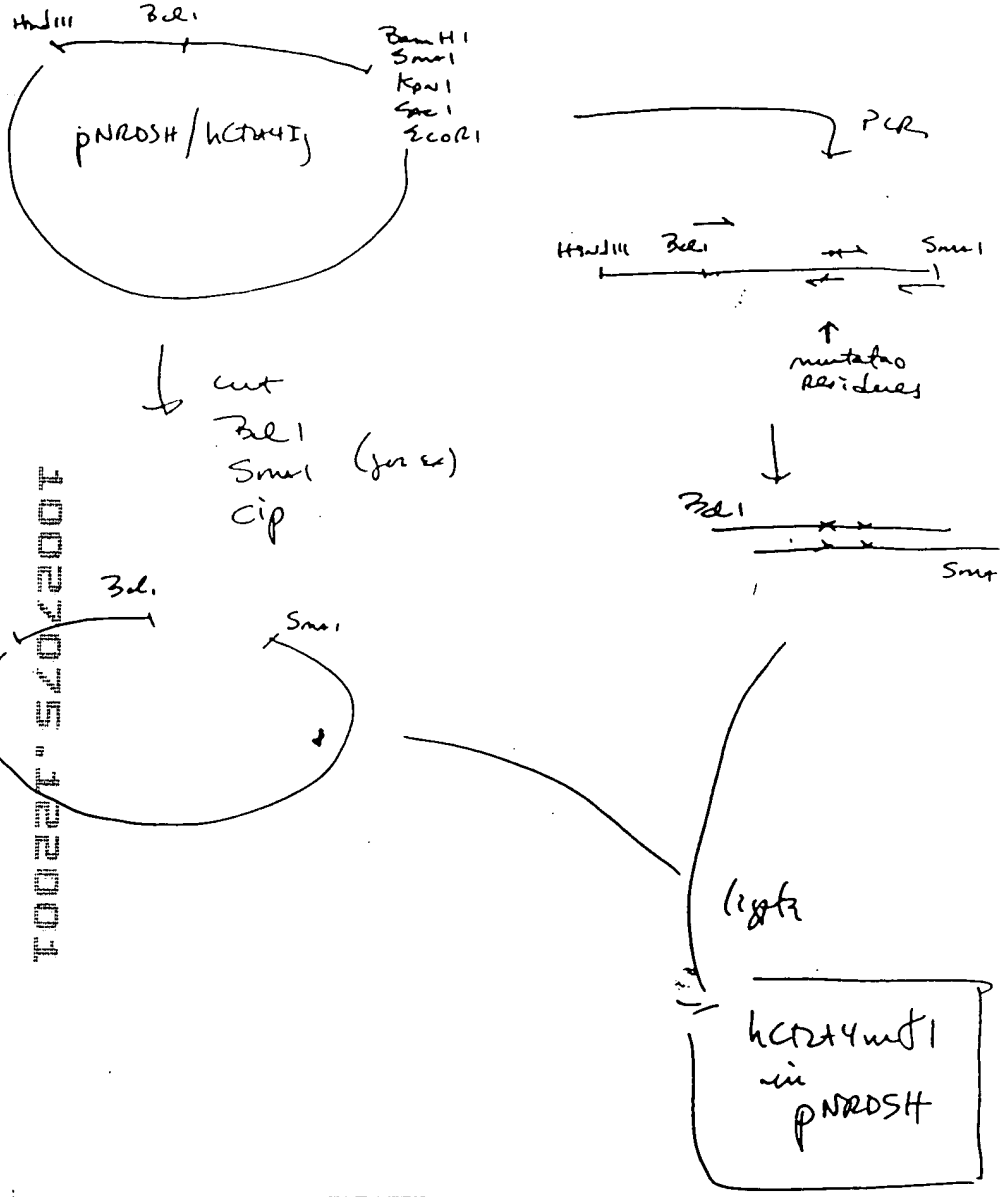
(Note that γ4 also lacks any ability to activate complement - S. Silver)



Read and understood by me

Date

Use NESTED PCR to generate a mutated 81 from [redacted] hCTL4Ij. Clone the mt81 back into hCTL4. [redacted] M pNRDSH:

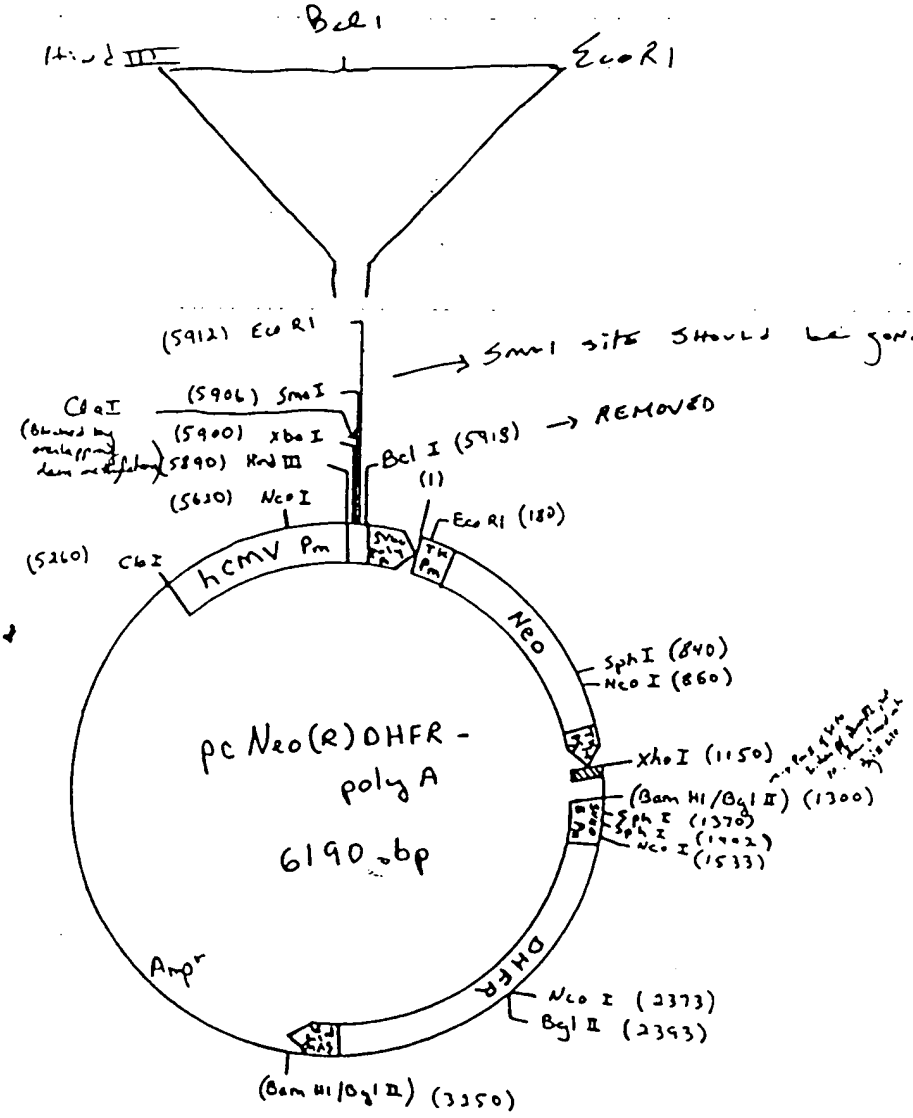


For this clone mutate residues as follows:

234	L	→ A
235	L	→ E
236	G	
237	G	→ A

Read and understood by me [redacted] Date [redacted]

Vector:



preproinsulin poly A

Enzymes that DO NOT CUT

EcoRV 1227 r3
SpeI 1227 r3
KpnI (1-9-92)

Read and understood by me

Date

FOIA 05-2004F

primers for [redacted] mutagenesis

for γ4:

5' primer - use G. Gray's original idea to knock out the cysteines in the hinge (γ4 has two)

P D (Q) E S K Y

BCL 1

5' GAG CAT TTT CCT GAT CAG GAG TCC AAA TAT

G P P S P S S P

GGT CCC CCA TCC CCA TCA TCC CCA

(G) (K) (P) (T)

GGT AAG CCA ACCC

DOUBLE CHECK THAT PNRDSH LACKS THIS restriction site

1st use this

3' primer

if needed still have these →

5' GCAGAG GAATTC GAG CTC GGTACCC GGGGATCC

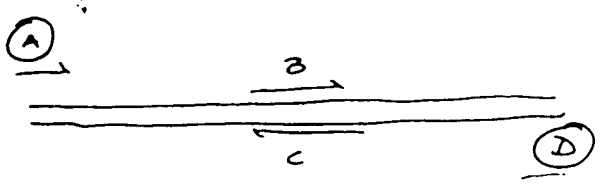
lock R1 Sac1 KpN1 Xma1 BamH1

CCAGTGTGGGG ACAG TGGG A CC CGCTCT GCC TCCC 3'

Read and understood by me

[redacted]

Date



5' primer ✓

(A): use Gary Gray's original γ_1 primer:

PRIMER

5' GAG CAT TTT CCT GAT CAG GAG CCC AAA TCT TCT CAC AAA ACT
P BglI a E P K S S D K T
CAC ACA TCT CCA CCG TCT CCA GGT AAG C — $Ig F_c$ —
H T S P P S P G K

— * — BamHI-SmaI-KpnI-SacI-EcoRI-ClaI-EcoRS- BglI —

— T7 promoter

3' primer (D): XmaI

BamHI SmaI KpnI SacI EcoRI

pSP72 MCS: 5' GGATCCCC GGGTAC CGAG CTC GAATTC 3'

3' CCTAGGGG CCCATGG CTCGAG CTTAAG 5'

PRIMER:

5' GCAGAG GAATTCGAG CTC GGTACCCGGGGATCC

lock

Read and understood by me

Date

10025275-10025307

1111

A-7

B and C:

L L G G P
5'C CTG GGG GGA CCG

A P E
(B) 5' CCATCTCTTCCTCAGCA CCT GAA GCT GAA GGG GCT
GCG GAG ... GCC
GCA ... GCA
GCG GCG

GAAGGAGTCGTGGACTTCGG CTCCCC CGT

P S V F L F P
CCG TCA GTC TTC CTCTTCCCCC 3'

GGCAGT CAG AAG GAG AAGGGGG G GTTTT GGG 5' (C)

FOIA 5-2007-008F

Oligonucleotide Requests:

DNA SYNTHESIS REQUEST FORM

REQUESTED BY Paul Bennett

PROJECT CHARGED B7 . 1GT

DATE REQUESTED .

DATE REQUIRED (NO ASAP)

SEQUENCE NAME muGAMMA4 - 5'

LENGTH 67

SEQUENCE:

pol I

5' GAGCATTTTCCTGATCAGGA
GTCCAAAATATGGTCCCCATT
CCCCATCATCCCCAGGTAAG
CCAAACC 3'

Read and understood by me

Date

Transient Expression of IgL h CTLA4 (3) Ig / FEIZ   A-8

→ 3F

293 culture supernatant tested again a IgG1, IgG4 ELISA using higher dilution.

Results:

DATE:

293 Transients

IDENTIFICATION	ug/mL IgG1	IgG4	Dilutions 1:10 → 1:2
ILL CTLA4 (+2) - γ1 1	2.12	1.77	↓
CTLA4 - mγ4 2	14.88	3.23	
IgL CTLA4 (+2) - γ1 3	34.26	33.65	
IgL CTLA4(3) - γ1 4	33.9	35.54	↓

⊕ Controls   2.25 μg/ml   156 ng/ml
expected: 2 μg/ml   125 ng/ml

These samples were tested for their ability to compete for B7 bin. Assay run by Nancy Green.

Optical Density

	1 (unlabelled CTLA4 Ig)	2 (IC samples #1)	3 (#2)	4 (#3)	5 (#4)	6 (WT)	7	8	9	10	11	12
70 μg/ml A	0.119	0.404	0.078	0.066	0.063	0.134	0.128	0.150	0.253	0.458		
25 B	0.170	0.412	0.083	0.083	0.075	0.128	0.147	0.182	0.291	0.343		
12.5 C	0.209	0.349	0.096	0.090	0.076	0.153	0.192	0.173	0.323	0.318		
31.25 D	0.287	0.412	0.138	0.104	0.096	0.199	0.237	0.233	0.448	0.398		
15.6 E	0.342	0.450	0.157	0.144	0.122	0.260	0.288	0.282	0.445	0.381		
7.8 F	0.390	0.454	0.268	0.158	0.149	0.285	0.368	0.349	0.549	0.415		
3.9 G	0.384	0.504	0.279	0.198	0.183	0.369	0.462	0.425	0.392	0.408		
0 H	0.425	0.649	0.407	0.462	0.524	0.597	0.496	0.489	0.523	0.424		

(max signal)

As before the IgL L CTLA4 is not functional. The two clones of IgL h CTLA4 do effectively compete w CTLA4-Ig-biotinylated.

Plasmids are ready for transfection into stable NS0 lines.

→ Samples titrated serially 1:2 - in 50 μl

→ All sample wells contain 50 μl of 70 ng/ml CTLA4 Ig biotin

→ 43

Read and understood by me   Date

10027075.122001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: Gary S. Gray, Jerry Carson, Kashi Javaherian, Paul D. Rennert and Sandra Silver

Serial No.: ~~09/227,595~~ Continuation of 09/227,595

Filed: ~~January 8, 1999~~ December 20, 2001

For: CTLA4-Cγ4 Fusion Proteins (as amended)

Attorney Docket No.: RPN-001

Group Art Unit: 1642

Examiner: Helms, L.

Assistant Commissioner for Patents
Washington, D.C. 20231

Certificate ~~of First Class Mailing~~ (37 CFR ~~1.8(a)~~ under 1.10)

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By: Larry Taylor
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~~Attorney for Applicants~~

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Sir:

We, Gary S. Gray, Jerry Carson, Kashi Javaherian, Paul D. Rennert And Sandra Silver declare:

1. We are the inventors of the subject matter described and claimed in the above-referenced patent application.

2. Prior to July 11, 1994, the invention described and claimed in the above-referenced application was completed in this country, as evidenced by the following:

A. Prior to July 11, 1994, we were in possession of a cloned CTLA4Ig molecule which was producing large amounts of active protein in CHO DG44 cells.

Page A-1 of Appendix A describes amino acid modifications to the Ig portion of the CTLA4Ig molecule. Deletion of the CH_2 domain from γ1 and mutations to amino acids 235 and 237 in γ4 are described.

Page A-2 of Appendix A describes amplification of the mutated γ4 Hinge-CH_2-CH_3 region and the cloning of the mutated γ4 into pNRDSH/hCTLA4 to replace the existing γ1 Hinge-CH_2-CH_3.

Page A-3 of Appendix A describes a second method which involves the use of nested PCR to generate a mutated γ1 (having an L to A substitution at amino acid 234, an L to E substitution at amino acid 235, and a G to A change at amino acid 237) from hCTLA4Ig and the cloning of the mutated γ1 back into hCTLA4 pNRDSH.

Pages A-5 and A-6 show primers for use in making mutated forms of CTLA4Ig.

The notebook pages submitted as pages A-1 through A-7 of Appendix A demonstrate that we completed the claimed invention in this country prior to July 11, 1994.

B. In addition, as shown on page A-8 of Appendix A, prior to July 11, 1994, we demonstrated that mutated forms of CTLA4Ig effectively competed with biotinylated CTLA4-Ig for binding to B7.
In the experiment presented at the bottom of the page B7Ig was put onto plates. Biotinylated CTLA4Ig was added to the plates in a fixed amount. Test forms of CTLA4Ig were added in serial dilutions to test for their ability to compete for binding to B7 on the plate. In this type of assay as the amount of an effective competitor is increased, the amount of biotinylated CTLA4Ig that binds decreases. This leads to a decrease in the optical density readout. Unlabelled CTLA4Ig (left row) was used as a positive control.
The data show that samples #2, #3, and #4 (in rows 2, 4, and 5, respectively) effectively competed with unmodified CTLA4Ig for binding to B7. As indicated in the table in the middle of the page, sample 2 was oncoCTLA4-mγ4; sample 3 was IgLCTLA4-γ1; and sample 4 was IgLCTLA4-γ1.

These data demonstrate that the modified CTLA4Ig molecules were still able to bind to B7.

Each of the dates deleted from pages A-1 through A-8 of Appendix A are prior to July 11, 1994.

We have been warned that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 or the United States Code, and that such willful and false statements may jeopardize the validity of the subject application or any patent resulting therefrom, and declare that all statements made of our own knowledge are true and that all statements made on information and belief are believed to be true.

Date: _____ Signed: _____

Gary S. Gray

Date: _____ Signed: _____

Jerry Carson

Date: _____ Signed: _____

Kashi Javaherian

Date: _____ Signed: _____

Paul D. Rennert

Date: October 16, 2001 Signed: Sandra Silver

Sandra Silver

10025705.112501

human - CD44 : Ig

STATUS : This clone, which is currently producing large amounts of active protein in CHOD44, is being methotrexate amplified.

RECENTLY focus has shifted to the Ig tail portion of this clone. Both Fc receptor and complement activation activities are determined by sequence in CH_2 domain.

REFS: Canfield + Morrison, 1991 J Exp Med (173) 14..
Jung et al, 1991 J Immunol. (147)
Tao et al, 1991 J Exp Med (173) 102
Duncan + Winter, 1988 Nature (332)

It would be useful to design a construct in which these Ig activities were eliminated.

This work will be exactly analogous to what was done last year for the antibody engineering project, where I deleted the CH_2 domain from γ, and mutated residues 235 and 237 in γ4.

See p 115, Book 1139 and //

See p 52, Book 1177 and //

Read and understood by me

Date

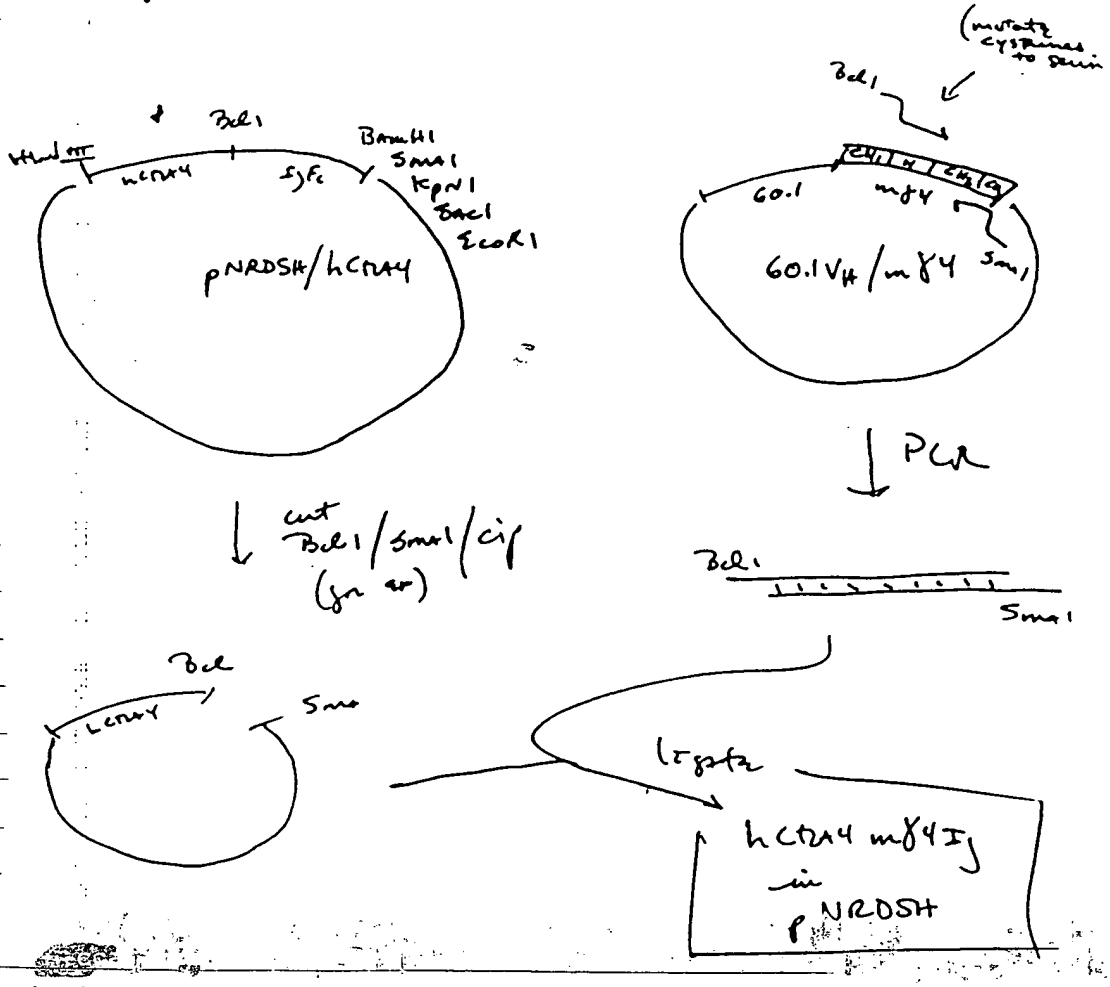
2 STRATEGIES will be USED:

hCγ4, mutagenesis of IgFc

possible strategies:

① PCR out the mutated γ4 H-CH_2-CH_3 region from 60.1 V_H and clone into pNRDSH/hCγ4 in place of the existing γ_1 H-CH_2-CH_3

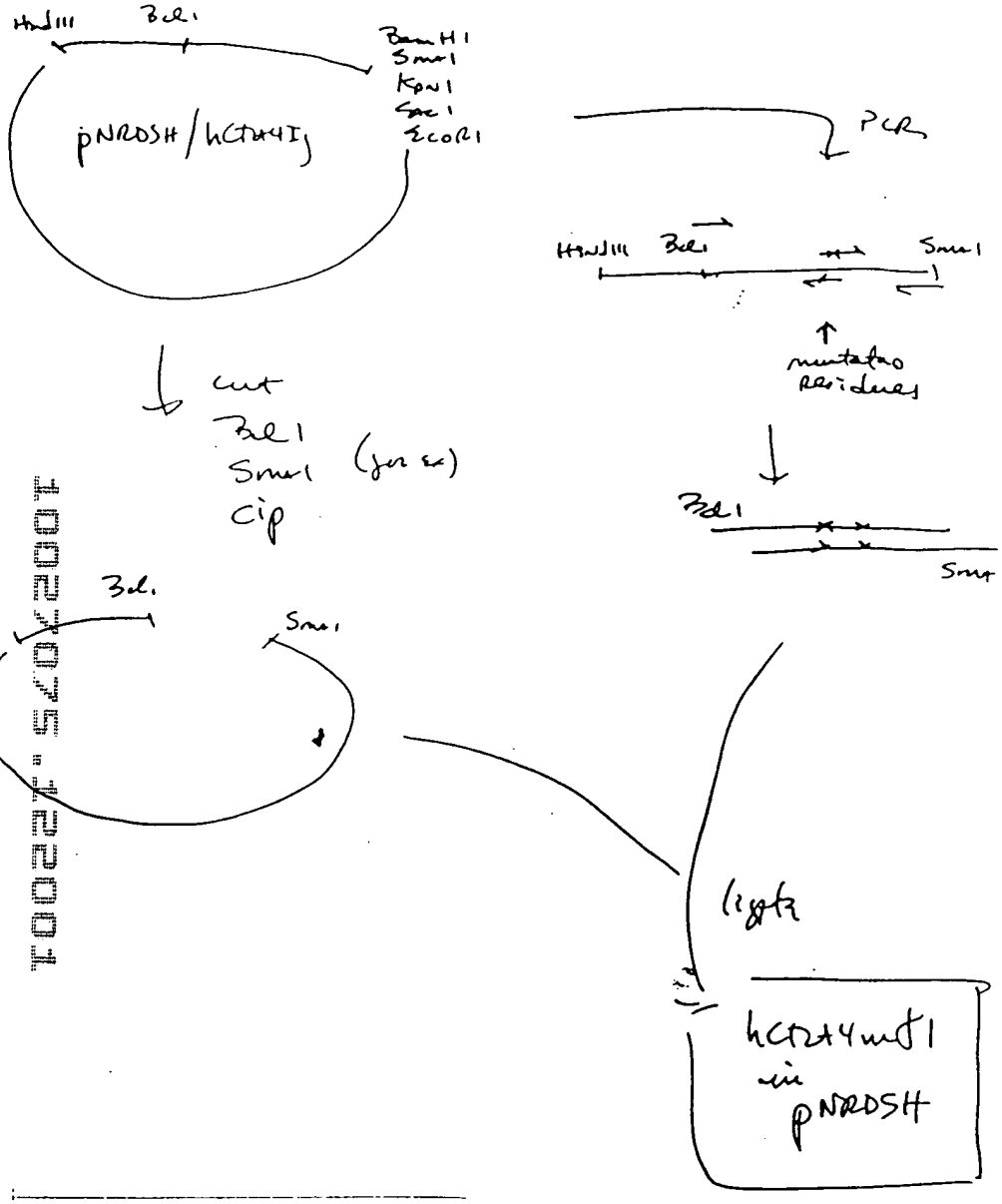
(Note that γ4 also lacks any ability to activate complement - S. Silver)



Read and understood by me

Date

USE NESTED PCR to generate a mutated 81 from [redacted] hCTLA4Ig. Clone the mt81 back into hCTLA4 [redacted] M pNRDSH:



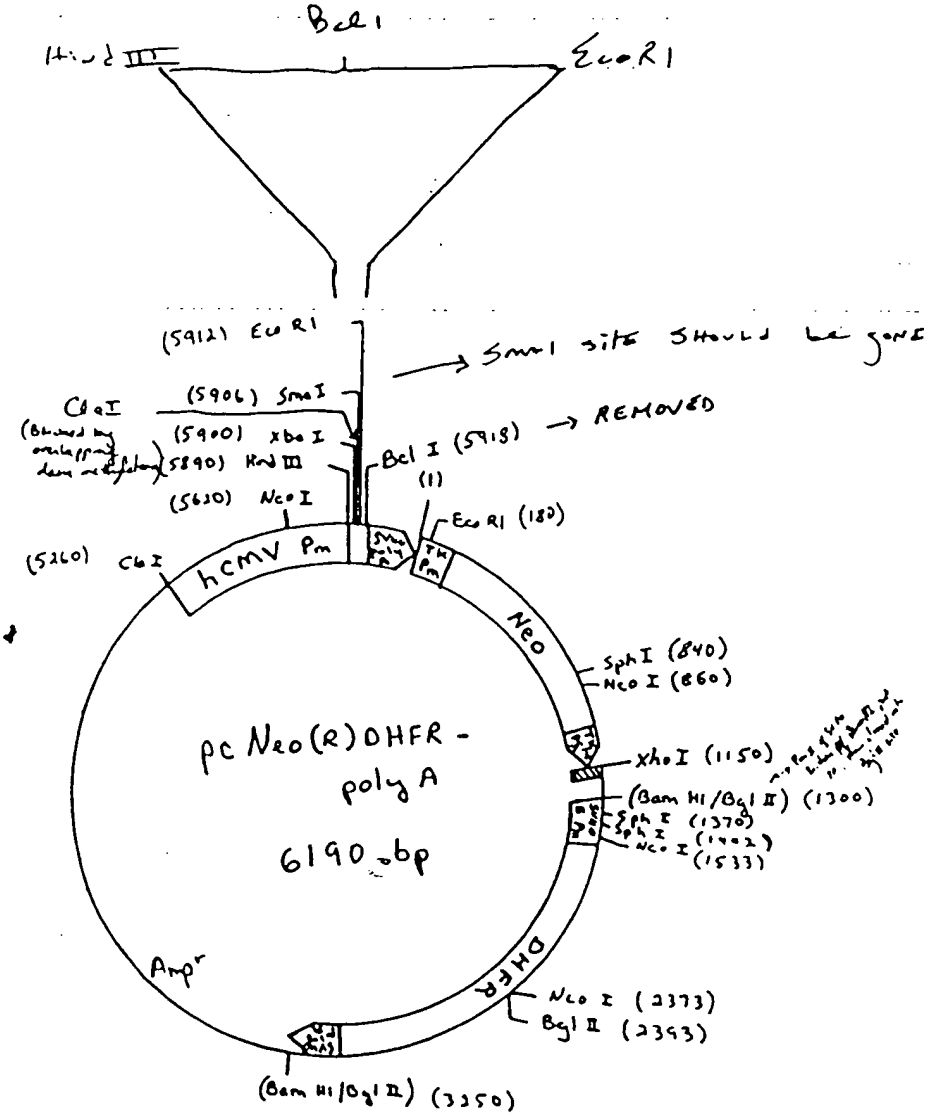
For this clone mutated residues as follows:

234	L	→ A
235	L	→ E
236	G	
237	G	→ A

Read and understood by me

Date

Vector:



Enzymes that DO NOT CUT

EcoRV 1227 p3
SpeI 1227 p3
KpnI (4.9.92)

Read and understood by me

Date

4002 5/20/2004

primers for [redacted] mutagenesis

for γ4:

5' primer - use G. Gray's original idea to knock out the cysteines in the hinge (γ4 has two)

```
                    P    D (Q)                          E    S    K    Y
                          BCL1
5'  GAG CAT TTT CCT GAT CAG GAG TCC AAA TAT
     G    P    P    S    P    S    S    P
     GGT CCC CCA TCC CCA TCA TCC CCA
     (G)  (K)  (P)  (T)
     GGT AAG CCA ACCC
```

DOUBLE CHECK THAT PNRDSH LACKS THIS restriction site

1st use this

3' primer

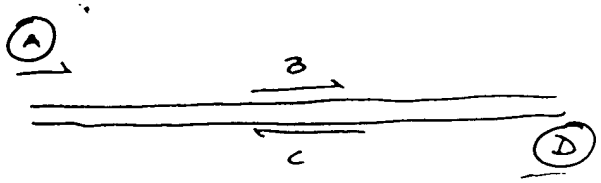
if needed still have these →

```
5'  GCAGAG GAATTC GAG CTC GGTACCC GGGGGATCC
    lock   R1       Sac1   KpN1   Xma1   BamH1
```

```
a4
me  CCAGTGTGGGG ACAG TGGG A CC CGCTCT GCC TCCC
                                              3'
```

Read and understood by me _____ Date _____

[redacted]



5' primer ✓

(A): use Gary Gray's original 5' primer:

PRIMER

5' GAG CAT TTT CCT GAT CAG GAG CCC AAA TCT TCT CAC AAA ACT
P BglI Q E P K S S D K T
CAC ACA TCT CCA CCG TCT CCA GGT AAG C — IgFc —
H T S P P S P G K

— * — BamHI-SmaI-KpnI-SacI-EcoRI-ClaI-EcoRS-BglI —

— T7 promoter

3' primer (D): XmaI

BamHI SmaI KpnI SacI EcoRI

pBR322 MCS: 5' GGATCCCC GGGTAC CGAG CTC GAATTC 3'

3' CCTAGGGG CCCATGG CTCGAG CTTAAG 5'

PRIMER:

5' GCAGAG GAATTCGAG CTC GGTACCCGGGGATCC

lock

Read and understood by me

Date

EXHIBIT 5 - 02/25/2004

A-7

B and C:

L L G G P
CTC CTG GGG GGA CCG

(B) 5' CCATCTCTTCCTCAGCA CCT GAA GCT GAA GGG GCT
GCG GAG ... GCC
GCA ... GCA
GCG GCG

GAAGGAGTCGTGGACTTCGG CTCCCC CGT

P S V F L F P
CCG TCA GTC TTC CTC TTC CCC C 3'

GGCAGT CAG AAG GAG AAG GGG G G TTT T GGG 5' (C)

Oligonucleotide Requests

DNA SYNTHESIS REQUEST FORM

REQUESTED BY Paul Bennett

PROJECT CHARGED B7 # 967

DATE REQUESTED .

DATE REQUIRED (NO ASAP)

SEQUENCE NAME MUGAMMA4 - 5'

LENGTH 67

SEQUENCE:

Pst I

5' GAGCATTTTCCTGATCAGGA
GTCCAAAATATGGTCCCCATT
CCCCATCATCCCCAGGTAAG
CCAAACCC 3'

Read and understood by me:

Date

Transient Expression of IgL hCTLA4-Ig / [illegible]

→ 38

293 culture supernatant tested again a IgG1, IgG4 ELISA using higher dilution.

Results:

DATE:

293 Transients

[illegible]	ug/mL IgG 1	IgG 4	Dilutions 1:10 → 1:2
IgL CTLA4(+2)-γ1 1	2.12	1.77	↓
[illegible] CTLA4-mγ4 2	14.88	3.23	
IgL CTLA4(-2)-γ1 3	34.26	33.65	
IgL CTLA4(3)-γ1 4	33.9	35.54	

⊕ Controls 2.25 μg/ml 156 ng/ml

expected: 2 μg/ml 125 ng/ml

These samples were tested for their ability to compete for B7 binding. Assay run by Nancy Greene.

Optical Density

	1 (unlabelled CTLA4Ig)	2 (JC samples #1)	3 (#2)	4 (#3)	5 (#4)	6 (WT [illegible])	7 (WT [illegible])	8 ([illegible])	9 ([illegible])	10 ([illegible])	11	12
50 μg/ml A	0.119	0.404	0.078	0.066	0.063	0.134	0.128	0.150	0.253	0.458		
25 B	0.170	0.412	0.083	0.083	0.075	0.128	0.147	0.182	0.291	0.343		
12.5 C	0.209	0.349	0.096	0.090	0.076	0.153	0.192	0.173	0.323	0.318		
31.25 D	0.287	0.412	0.138	0.104	0.096	0.199	0.237	0.233	0.448	0.398		
5.6 E	0.342	0.450	0.157	0.144	0.122	0.260	0.288	0.282	0.445	0.381		
7.8 F	0.390	0.454	0.268	0.158	0.149	0.285	0.368	0.349	0.549	0.415		
3.9 G	0.384	0.504	0.279	0.198	0.183	0.369	0.462	0.425	0.392	0.408		
0 H	0.425	0.649	0.407	0.462	0.524	0.597	0.496	0.489	0.523	0.424		

(no x [illegible] signal — [illegible])

As before the IgL hCTLA4 is not functional. The two clones of IgL hCTLA4 do effectively compete w CTLA4-Ig-biotinylated.

Plasmids are ready for transfection into stable NSO lines.

→ Samples titrated serially 1:2 - in 50 uL

→ All sample wells contain 50 of 70 ng/ml CTLA4 Ig biotin

→ 43

Read and understood by me

Date